

First things first in the Third World

AS THE national papers pile up in the headquarters of the United Nations Conference on Science and Technology for Development due now in less than a year's time, the pace of international meetings preparatory to the conference also grows. One of the most interesting, because of its wide ranging geographical representation, particularly of socialist countries, has been that organised by the World Federation of Scientific Workers in conjunction with the Union of Algerian Engineers and held in Algiers last week. The gathering, the responsibility of Professor J. M. Legay, showed rather clearly both the strengths and weaknesses of calling an international meeting to discuss a subject so large and so open to wide differences in interpretation.

Let us dispose of the weaknesses rapidly in order to concentrate on the strengths. The contribution to the conference by members of developed socialist countries was largely disappointing. Many from other parts of the world must have looked forward to discussion of socialist policies on development; they were given only vague theorising and totally uncritical self-advertisement in papers of almost uniform tedium. Example "The mission of structuring the overall problematique of social development into development complexes is to facilitate decisions in situations when concrete variants of sectoral development have to be derived from strategic goals or generally formulated concepts".

If there is one message that ought to get back to the governments of socialist countries—and indeed of many capitalist ones too—it is that the preparations for UNCSTD are making many scientists and technologists of the developing world much more critical of the statements of developed nations, and meaningless, self-satisfied or jargon-ridden papers will cut little ice.

That said, it is pleasing to be able to report that contributors from developing countries, and a few non-socialist countries, did have some significant and provocative things to present. It was clearly a hope of the organisers that the meeting would shed some light on scientists' responsibilities in the establishment of the New International Economic Order, that concept which also emerged from Algeria just five years ago and which is espoused with enthusiasm throughout much of the Third World as a way, *inter alia*, of obtaining control over natural resources and exerting more influence on the global monetary system. This new order is, however, not mentioned much in the capitalist world; as Professor E. H. S. Burhop, president of the Federation, put it, "the

overwhelming majority . . . in the industrialised capitalist countries . . . have never heard of the new order and are quite unprepared for the vast economic changes in their own countries it implies". The contributions that made the most impact, however, were not those that concentrated on praising the new order, or for that matter reviling the multinational corporations. Most effective were those papers that took a hard look at real problems in the developing world, admitted mistakes rather than tried to dole out blame, and made positive proposals for the alleviation of difficulties.

Two issues of importance seemed to emerge. The first was the very serious problem of effective use of intellectual resources in the developing world. Higher education in the Third World is easily seen as a status symbol, by governments and people alike. The more universities, the more PhDs, the closer the resemblance to western seats of learning, the better. But the further this path is trodden, the less it seems that trained scientists and technologists have an understanding of what people's needs are. This, of course, is not a phenomenon exclusive to the developing world, but whereas in the developed world we may have the luxury of debate as to how "relevant" university education need be, such a discussion in the developing world must surely be subservient to very visible national needs. And yet India, with more than a million technically qualified people, hardly seems to have harnessed this resource at all, and, in the words of a Pakistani participant, the role of science in the development of his country "has not been very significant".

There are many factors that have apparently contributed to a detachment of education from urgent national needs in many developing countries. The siren-call of developed-world science diverts too many into vain attempts at imitation. Poor experimental facilities drive many a good scientist into rather sterile theory. Service to the community through the provision of better water supplies or more efficient energy use is not seen to be very prestigious. There is a pressing need for those concerned with national science and educational policies to recognise that higher education and scientific research establishments can best serve development not by mimicry of the developed world but by a long cool consideration of local, often rural conditions where the potential for science-based improvements is still enormous.

The second issue of importance to come from the conference was that development which simply results in more



jobless cannot be acceptable. Higher productivity can well lead to unemployment, so it is most important that the aim, particularly within the rural sector, should not be for a mighty technological revolution which releases vast numbers of agricultural workers to flock to cities incapable of receiving them. The abundant manpower resources of many developing countries have to be handled with the greatest of care. One Bangladeshi contribution put this very succinctly: "in most cases our object is to upgrade simple rural technology to a slightly higher level of sophistication which improves the quality of life for the workers and creates more job opportunities." The emphasis, then, is on modest aims and on evolutionary development. In a notably thoughtful contribution, Dr H. Harada, Deputy Assistant Director-General for Science at Unesco, drew attention to the need to accept a slow and deliberate pace, in which a nation is at pains to develop science ahead of technology.

So what comes from such a conference? A final docu-

ment, necessarily general, of course. But maybe the germs of something more substantial. Numerous participants, while recognising that no New International Economic Order will come about without a correspondingly new national order, note nonetheless that there is enormous scope for international research centres to cope with developing world problems. Few of these problems respect national frontiers, nor are they such that questions of commercial secrecy should be involved. If there can be an International Centre for Theoretical Physics, specifically aimed to assist developing countries, is there not some sense in international, maybe regional centres on other scientific matters perhaps of a more immediate concern to development? A few exist already, of course, notably through the Canadian initiative of the International Development Research Centre, but there was little doubt in people's minds at Algiers that there was scope for more. As David Dickson reports on the following page this mood now seems widespread. □

Multinationals and development

Multinational corporations often come under criticism for their involvement in the developing world. But, argues Lewis Branscomb, Vice President and Chief Scientist of IBM, multinationals make major contributions to indigenous development.

THE United Nations Conference on Science and Technology for Development (UNCSTD) will look at the urgent need for indigenous capabilities in both developed and developing nations to mobilise science and technology in the cause of human problems. Poorer nations are understandably impatient with the slow and expensive process of institution building out of which emerged the education, research and industrial enterprises of the industrial democracies. But without the capacity to absorb, adapt and exploit technical knowledge, no amount of documented knowledge, patent licences or computer access will produce the economic results that are needed. How can this capacity be built?

National assistance agencies, international banks and private foundations can do a lot more than they have. Technical assistance programmes should be separated from security and capital assistance. Every prosperous nation has to find its own way to focus the energies of its universities and private sector establishments on institution building in cooperation with less developed countries. President Carter has made a farsighted proposal in his speech in Venezuela, proposing a government-sponsored foundation for technical cooperation to deal with this long-term need of the developing world.

Technical cooperation in education and research can look after many basic human needs, but industrial capability is only built up very slowly in this way. What is a more direct route? The fastest way to transfer technical capability from one nation to another is by exchange of the people with skills and experience, tools and procedures, and (when required) their financial resources and foreign market access. American industrial technology was built by direct foreign investment by European firms in Victorian times. Research was little known in 19th century American universities; the nation's industry was craft-oriented. Most of the population was in agriculture, which based its productivity more on the expanse of unexploited fertile land than on the cropping technology of Europe.

Why then are today's industrial enterprises which are

engaged in technologically-sophisticated operations in many countries (the "multinationals") so often regarded as part of the problem, when in fact they are a part of the solution?

When a responsible, modern multinational starts a business operation in a host developing country, it contributes to indigenous technical and managerial development in many ways. For example:

- By hiring and training host-country nationals for all levels of employment in the national subsidiary. Education and experience in industrial operations is gained in the community and is made successful under local conditions.
- Through technical cooperation of the multinational's local manufacturing plants with its plants and laboratories in other nations, a broad base of technical support is available in the event of technical difficulties.
- By obtaining materials, parts and assembly from local suppliers (IBM has 80,000 such vendors worldwide), a great deal of technological knowledge and experience is transferred, as the local company of the multinational works with each vendor to assure quality control of the product.
- As local skills develop and local markets justify expanded investment, more complex products can be undertaken by national subsidiaries and access to international markets expanded. In this way multinationals make important contributions to the balance of trade in many developing countries.
- Many products, such as communications equipment, trucks, machine tools and computers, not only contribute to national wealth directly but have large secondary benefits because their use in other sectors can increase productivity in the economy as a whole.

Sovereign nations must certainly look after the interests of their citizens by determining the rules regarding investments of skills and money within their borders. It is important that the leaders of those countries avail themselves of technical advice from those with industrial experience, to ensure that policies driven by economic objectives take into account technological realities. Transferring technology, even within the component parts of a single American or European enterprise, is not easy and requires time, training and extra expense. But above all it calls for a cooperative attitude by all the participants.

Let us hope that this is the outcome of the 1979 conference. □

The United Nations conference on Technical Cooperation between Developing Countries, which ended in Buenos Aires (right) last week, was a great success for third world countries, writes **David Dickson**

Camera Press

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Developing countries to boost joint research

JOINT research projects between two or more developing countries are to receive greater emphasis, according to an ambitious 'plan of action' adopted last week by 138 member countries of the United Nations Development Programme. The plan was the main result of the conference on technical co-operation between developing countries (TCDC) which ended last week in Buenos Aires, Argentina.

The plan of action is based largely on suggestions made to the conference by the so-called 'Group of 77' — comprised now of 114 developing nations. The plan suggested that developing countries should "encourage existing national research and training centres to broaden their scope of activities to include programmes and projects which are of interest to several countries at a sub-regional, regional, and inter-regional level".

The conference also accepted a resolution submitted by Jamaica on behalf of the Group of 77 urging all developing countries to cooperate in strengthening existing research and training centres, with the intention of giving them "a multinational scope in the framework of TCDC" and where necessary establishing new institutions to achieve these ends.

And in order to prevent the plan of action being shelved as a well-meaning but ineffective document, the conference included in its recommendations specific directions for the way in which TCDC is to be encouraged and propagated, both within and outside the UN system.

Thus while rejecting as unnecessarily bureaucratic the idea of a new UN body with specific responsibilities for TCDC issues, the plan of action directs the UNDP to organise regular meetings—initially annual, subsequently biannual—or the planning ministers of member states to discuss progress towards TCDC goals.

Significantly the plan indicates that funds currently allocated by UNDP for regional use should now be devoted "to the maximum extent possible" to TCDC projects. Funds allocated to inter-regional or global projects could be used for TCDC projects proposed

by two or more countries from different regions.

The significance of these decisions is that, while using international funds, the developing countries will be able to exert greater control over the way that money for technical projects is spent rather than the major impetus for the use of such funds coming from the developed countries.

Many delegates to the conference felt that the agreement on a regular meeting of planning ministers and the shift in the balance of control over technical projects had resulted in greater success for the conference than had previously been expected.

"The consensus that the conference was able to achieve on the plan of action represents a major victory for the developing countries", said Mr Abdel Razzak Meguid, Minister of Planning for Egypt and chairman of the main committee of the conference secretariat, in protest at the weakness of proposals put forward in an earlier draft of the plan.

"We have succeeded in making it acceptable to the North that it is in their interests to strengthen the 'horizontal' links between developing nations. That bridge is now not only accepted as a hope, but as a necessity if the world community is to survive".

However, few speakers at the conference pretended that TCDC was a new strategy. Background papers prepared for the conference, for example, described the complex network of overland trade routes that had existed between different countries in Africa before the arrival of European traders and colonialists.

Furthermore there has in recent years been a steady growth in joint research projects between various developing countries. The University of Chile, for example, is now co-ordinating a Latin American scheme for co-operation in postgraduate teaching and research in the biological sciences, which has already set up studies in areas such as the spread of plant viruses and the biological effects of working at high altitudes (such as in the mines of Peru).

What many delegates felt was new,

however, was the emphasis that the conference was able to place on the concept of TCDC not merely as defined by a limited set of bilateral or multi-lateral agreements, but as a basic strategy for achieving the goals of what has come to be known as the 'new economic order' and the joint attempts of developing countries to counter the economic dominance of the developing world.

Thus initially the conference had been conceived in relatively narrow terms as dealing primarily with issues relating to technological co-operation, partly at the instigation of the more advanced of the developing nations, many of whom—such as the host country Argentina—are looking for new outlets for their goods and technical services.

However, during the evolution of the conference, the concept of TCDC has gradually become much broader, covering radical approaches to education and research, through to strengthening transportation, communication and information links among developing countries.

"Since agreeing in principle on the need for a 'new economic order' in 1974 the United Nations has not done a single thing to translate a programme of action into an actionable programme. But I think that this conference has laid the framework for such an actionable programme", Mr Meguid said after the plan had been accepted by the conference.

Other delegates were similarly confident that the plan, which will now be placed before the General Assembly of the United Nations, provides a firm basis for a substantial shift in control over the content of development programmes from the developed to the developing nations, and that the regular meeting of planning ministers will provide considerable support for this trend.

As far as scientific research is concerned, the conference did not consider in detail any particular programmes or list of priorities, and many of the participants from specialised UN agencies such as the World Health Organisation and the Food and Agri-

cultural Organisation had to remain primarily as observers—much to the frustration of some who claimed considerable practical experience in the issues under discussion.

However, the plan of action makes a number of suggestions about how research should be conceived within the TCDC framework. For example, it speaks of the need for developing countries to combine research efforts

and share results “both with one another and with other developing countries by means of agreements on scientific and technological co-operation, strengthening national design, national laboratories, research centres and scientific and other institutions”.

Putting flesh on these ideals will be one of the main objectives of next year's United Nations Conference on Science and Technology for Develop-

ment, which will take place in Vienna. But many delegates left Buenos Aires with the feeling that despite internal differences, the developing countries have found in the issue of technical co-operation a way in which they can work together in presenting concrete demands to the developed world, and that this in itself was a major step in bringing the new economic order closer to reality. □

US geneticists look to Europe for research facilities

RESEARCHERS at Genentech Inc, San Francisco, claimed at a Munich conference last week that they have induced bacteria to synthesise the two separate amino acid chains of human insulin—and to have combined these chains to make minute quantities of the hormone. Last year, the same team used a similar technique to induce bacteria to produce the hormone, somatostatin, and earlier this year, another team led by Walter Gilbert of Harvard University announced that they had persuaded bacteria to produce rat proinsulin attached to the bacterial enzyme penicillinase.

Synthesis of the artificial insulin gene used by Genentech began at City of Hope Medical Centre, California late last year. Researchers there synthesised artificial genes consisting of the DNA sequence corresponding to the amino acid sequence of the A and B strands of human insulin. Six months later, they turned the synthetic genes over to David Goeddel and Dennis Kleid, biochemists at Genentech.

Goeddel and Kleid inserted the synthetic genes for the two strands of insulin into plasmids which were then introduced separately into *Escherichia coli*. The bacteria replicated and expressed the genes as the A and B amino acid chains. These were then mixed to produce a fraction of a milligram of insulin which, it is claimed, is similar to human insulin.

Biological tests for the activity of insulin, however, have yet to be done. Even though chemical tests and radio-immunoassay have indicated the presence of the molecule, some researchers question the claim that the insulin produced is the chemical equivalent of human insulin. This is because radio-immunoassay cannot distinguish between different insulin varieties. Also, the B-peptide chains produced during the recent work are impure, and some researchers feel that the amino acid analyses conducted so far may be insufficient to establish the presence of

the desired sequence.

The development of a new source of insulin for the treatment of diabetes has great practical importance. About 5% of diabetics are allergic to the animal insulin now used, and each patient requires 10 bovine pancreases per year at a cost of more than \$100. Bacterially produced insulin should eliminate allergic reactions and may be cheaper and easier to supply than animal insulin. Before the newly produced insulin could be marketed, however, it would have to undergo extensive animal and human tests which could take two to five years, though Genentech president Robert Swanson foresees no real difficulties in scaling up the procedure and passing the tests.

Such rapid success in developing a practical application of a technique raises a host of questions. The technique was discovered so recently that the original patent application has not yet been processed, and neither have revised safety regulations been implemented. Already the race for other commercial applications is hotly contested. New patents have been applied for, the breadth of the original patent remains unclear, and several leading American researchers are reportedly setting up shop in Europe to escape regulatory delays.

The original research leading to the procedure used in synthesising artificial genes was conducted with funds from the National Cancer Institute, the

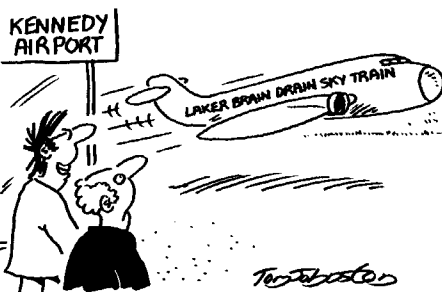
National Science Foundation, the National Institutes of Health, the University of California and Stanford University. All parties eventually agreed that NIH would enter an institutional patent agreement with Stanford University and the University of California. Stanford would administer licensing of the technology, under terms of the agreement and share royalties equally with the University of California. After public hearings last spring, NIH granted Stanford permission to go ahead with licensing, provided any company granted a license would abide by NIH safety guidelines for genetic engineering.

Niels Reimers, Stanford's manager of technology licensing says he expects a patent to be issued within a matter of months, and believes it will cover much of the basic technique for producing recombinant DNA in *E. coli*. Stanford will probably wait until the patent is actually issued to begin licensing.

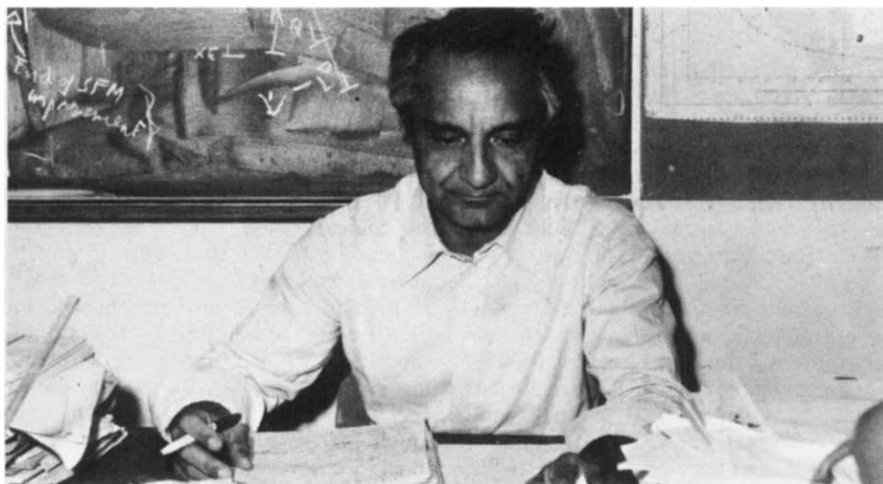
Such a patent would apply only in the United States. For that reason, and because of early stringent regulations, some scientists who are trying to create insulin by isolating and transferring a human gene have gone to Europe to work. Applications to use the country's first super-secure “P-4” facility, at Fort Dietrick, Maryland, will not be processed until later in the autumn. New, less stringent safety guidelines will also not go into effect until later this year.

William Rutter, a molecular biologist at the University of California, San Francisco, is already looking to Europe as the place to continue his work on the human gene for insulin. “One is forced to do that if one wants to do the experiments” he says. His group is planning to work in France. The potential advantage of using the natural gene for insulin, he says, is that it includes additional information for folding and joining the A and B peptide strands.

John Douglas



“Yes, we go both ways now”



Antonino Zichichi: shy of the camera, but not of politicians

Sicily's scientific evangelist

The European Physical Society, which meets next week in York, has for its president a Sicilian very powerful in Italian science politics: Professor Antonino Zichichi. He spoke recently to **Robert Walgate**

ANTONINO Zichichi, 48-year-old son of a Sicilian small businessman, entered university without knowing that physics could be a career. Now he is Professor of Advanced Physics at the University of Bologna. He is also virtually king of Erice, a Sicilian hill-top village where 15 years ago he established a summer school: the Ettore Majorana Centre. (A hundred scientists a week now visit Erice. "I had left Sicily. I felt guilty. I wanted to help.") Zichichi has also helped the Italian National Nuclear Physics Institute (INFN), of which he is president. Earlier this month he succeeded in doubling its budget, committing Italy to three new accelerators: an electron storage ring, a superconducting cyclotron, and an electron linac.

Professor Zichichi is a particle physicist who keeps company with the Italian ministers of science and education. And he is now President of the European Physical Society. He is also, without doubt, a thoroughgoing romantic. He is romantic about Europe. He is romantic about science. But both romances are tinged with realism: he wants to get things done.

'Culture' is an important word in his vocabulary. By it he means largely disseminated 'pure science'. Technologists, he says, are "culturally 300 years in the past; they should understand quantum electrodynamics". According to Zichichi, scientists create "new culture". "The greatest three things that mankind has created are first language, then logic, and finally science". Language needs very little logic, just enough for internal consistency. Logic,

invented 2,000 years ago, "is above language; it is something new." The most rigorous form of logic is mathematics; but you can invent anything in it, such as spaces of 25 dimensions. Science, which began with Galileo, is the logic of nature. "Science tells you if you want to believe something just ask nature. It took 2,000 years to learn that force was proportional to acceleration and not to velocity, because the Greeks only argued". Thus Zichichi believes that science is the highest achievement of mankind.

"But science has no impact in human culture. Human culture is still dominated by language. People can still be influenced by a slogan. Invent a slogan and you will have millions of people following you. A slogan is an insult to human intelligence." Science, says Zichichi, "has produced a lot of science but very little culture." What would be poetry "if poets had kept their work only in a closed drawer, or communicated only among themselves? Or painting if Raphael or Picasso had kept their paintings locked away? Or music if musicians had played only to each other? With science it's the same story. There are immense treasuries of knowledge locked away in our laboratories and our libraries, but scientists have done very little to make them known to everybody."

There is a barrier to cross in that one has to work to understand science; but 300,000 years ago probably very few people had language. Using a language is not easy; but now everyone can speak. Why? Because we hear

speech from birth. "My conviction is that the same should happen with science. One day everybody will be scientists—not in the sense that they will make discoveries, but they will understand the discoveries that others are making, which is already a very great step forward".

Professor Zichichi is not unaware of the more temporal benefits of publicity. I was interviewing him at CERN, the European nuclear physics laboratory near Geneva, where he has spent most of his career since 1956. I asked him what he considered his greatest contribution to physics. It was the discovery of the antideuteron—a nucleus consisting of an antiproton and an antineutron—which he made in one night in 1965. "If you forgot the accepted wisdom about invariance principles and just thought classically, you could ask who told us that the antiproton and the antineutron stuck together like the proton and the neutron? And if they did not, the whole route to the antinuclei would have been stopped." Zichichi found the antideuteron. "And we didn't make any press release—nothing." Seven years later another group rediscovered it and published it widely. "At this point I decided to take more care of public impact".

Zichichi began to write for an Italian newspaper — "the greatest", *Corriere della Sera*, in a column titled 'new culture'. "I don't like to call it science popularisation". After two years he moved to *Il Giornale Nuovo*, to reach a new public, and now he writes articles of up to 1,000 words for the Saturday edition of the Rome newspaper *Il Tempo*. The latter has a circulation of 300,000, half that of his first paper, but it is read by "political people, religious people, in Rome". It is therefore more influential; and Zichichi gets more letters about his *Il Tempo* articles than any others.

If communicating science is one of Zichichi's passions, then so also are people. He sees European Physical Society first in terms of creating friendships; he sees his negotiations with ministers in terms of relationships and conversations, as a matter of convincing individuals face-to-face with his arguments. "I like to leave the administration of INFN to those who can do it better than me. I work on the scientific strategy; I try to convince people at the highest level with good arguments... Politicians are not stupid people. They are very smart. Too many of my colleagues make the mistake of thinking them stupid."

I asked Zichichi what was the place of a scientist in Italian politics:

"Nonexistent."

"But you have a lot of influence."

"I am the exception not the rule."

"How do you do it?"

"I don't know how I do it. I think it's because they understand me and not the others."

And yet "I don't like to waste my time with politics. I took the presidency of INFN because I thought we were going to have troubles. The first thing I like to do is the research I am interested in. But you cannot spend your life complaining . . . some time ago I was arguing about strategy and so on with my colleagues in INFN, and at a certain point they said well, you cannot refuse to be president if you really want to go on like this. I would have preferred someone else to step in; but I realised I had to accept responsibility for the things I wanted to get done."

"Do you have any other administrative ambitions?"

"No. Zero. Absolutely no." I asked about CERN. "In CERN I do only physics. I've never had any responsibility of any kind. And I'm very glad, for there must be a place where you do only physics." Could CERN be better run? He was not in a position to say. "It is always easy to criticise organisations. I have a positive attitude to anything existing. But what I can tell you is that it is easy for big organisations to slip out of purely scientific control. I am facing this problem at INFN, and that is only one-tenth the size of CERN."

"Would you like to be director of CERN?"

"No. Because if you are director of CERN you cannot do any more physics. And I want to do physics. It is all right for a theorist. But if you are an experimenter you must make a choice: either you are director of CERN or you do physics. The games I am in now, I can combine with my research; I would feel without an important part of myself if I had to give up physics."

Erice perhaps weighs equally with research in Zichichi's constitution: "I am in touch by telephone for a few minutes every day". Why did he start the summer school? For a complex of reasons. "As you know Sicily is a place of great culture. Pretty well every culture but the Chinese has been through Sicily. But it is an underdeveloped part of Europe. I was not living there, and in a certain sense I felt guilty. But what could I do?"

"One thing was clear to me. The universities started 1,000 years ago because people wanted to know what was going on at the frontier or knowledge. Books were very slow. So people had to meet. But now universities cannot bring people to the frontier because of the great development of modern knowledge. So the old university spirit has been lost.

"People still want to be brought to the frontier. But who is going to teach you? It may be that at X you have the greatest world specialist in some discipline and you are lucky; but you miss the other disciplines. So if you can establish a place where people can be brought to the frontier of various fields then they will come. That is the principle of the Ettore Majorana Centre at Erice."

The school is financed by the Sicilian Government (Sicily is independent of Italy but for foreign policy), by INFN, and occasionally by other bodies such as NATO. When it started Zichichi had offers of buildings. "But underdeveloped countries are strewn with cathedrals in the desert. I wanted to be sure this place would work. And Erice beautiful, of human dimensions."



Erice, home of Zichichi's summer school.

Erice is built on a volcanic outcrop of rock, like a natural fortress. On one street you walk on the same stones as the Romans; more recently it was like a Switzerland in Sicily, housing and protecting the richest families. Zichichi's office is the highest part of Erice. "The view from there is absolutely fantastic." The 'summer' school (it lasts from March to December) is the whole economy of the town, and, according to one enthusiastic visitor the few inhabitants (some 100 or so, says Zichichi) bow their heads to Zichichi as he passes.

Zichichi hands out prizes to the best students (in fistfuls of Lire at the end of a course, an ex-student says) "but it is not easy to win a prize. The people who go there are really very brilliant. It is a pleasure to be there."

In March this year Zichichi 'emerged' as President of the European Physical Society, through a similar process to the one that found him president of the INFN: he wanted to do things. Following in the footsteps of his predecessor—and father-in-law—Gilberto Bernardini he has a romantic ideal for the EPS, uniting East and West and forging links among nations: "We should contribute to the cultural

unification of Europe."

Zichichi also sees the EPS as a source of "one of the greatest human feelings", friendship. "In the present age when there is an enormous amount of communication and we know what is going on on the other side of the world, we don't know our neighbours two metres away. And it is very important for physicists to talk, to get to know each other."

The EPS should also play a role in the five great physics projects in Europe: a giant proton synchrotron (probably in the USSR); a very large electron-positron colliding beam machine; JET; a large synchrotron radiation laboratory; and a millimetre wave radio interferometer. It should also develop proposals on the physics components of two new teaching institutions at present under discussion in Europe: a European 'science university' and a 'Mediterranean polytechnic' for the underdeveloped regions of the Mediterranean. And it should encourage the exchange of students and professors, perhaps for whole periods of a year's teaching: "imagine the impact of a London professor of quantum mechanics teaching in say Palermo, or Leningrad, for a whole year. Sabbaticals are not enough. We need to interest governments in this."

In the end Zichichi will always come back to his physics. He is an unconventional researcher. He was the first to look at the production of leptons in collisions of hadrons, work which eventually led to a Nobel Prize for Samuel Ting in the discovery of the 'hidden charm' particle J/ψ . "Ting mentioned me in his Nobel address as the pioneer. I can be proud and ashamed—proud that I started, and ashamed that I gave up". Also he spent five years searching for a lepton heavier than the muon at Frascati, by looking at electron annihilations producing an electron, a muon, and anything—the same reaction in which Martin Perl at Stanford discovered the 1.7 GeV tau lepton ten years later (the tau was just above the Frascati energy, like the J/ψ itself. There is little doubt that Frascati's energy was very unfortunately chosen.)

Now Zichichi is "trying to break the proton". The concept that the quarks are infinitely bound into the proton "is only at the level of 'theoretical feeling'" says Zichichi. "There is no proved theorem that says they should stay in there." Zichichi, while admiring elegant theory, has no truck with theoretical speculation, any more than he has time for reading the fantasies of novelists. In the matter of the proton he wants to ask Nature her logic. He has, as he puts it, "a very realistic view".

news and views

Cholera and related diarrhoeas ('turista')

from Richard A. Finkelstein and Mary Boesman-Finkelstein

CHOLERA and the related diarrhoeal diseases extract an incalculable global toll in human misery, morbidity and mortality. They also have a direct impact on human nutrition and growth and affect world food supplies through their effect on livestock. On August 6–11 they were the subject of the 43rd Nobel Symposium* at which 30 experts discussed the epidemiology, molecular biology, immunology, pathophysiology and significance of the enterotoxigenic enteropathies as a worldwide health problem. The symposium could not have been more timely; as a result of the tremendous progress during the past decade, there was a lot to be said.

Of the many highlights of the symposium, the dramatic advances in the treatment of cholera and related diarrhoeas, the dynamic interaction between diarrhoeal disease and malnutrition, developments in understanding the pathogenesis of diarrhoea at the molecular level, and the imminence of a living attenuated vaccine for cholera and the immunologically related enterotoxigenic enteropathies are certainly worthy of further brief review.

The remarkable developments in therapy of cholera and related diarrhoeal diseases pre-eminently illustrate the practical rewards of basic research. The results would certainly have been gratifying to the late Dr Robert Allan Phillips who, were he alive, would have been a key figure at this conference, as it was he who pioneered both intravenous and oral fluid and electrolyte therapy. His observation (Phillips *Fed. Proc.* 23, 705; 1964) that the incorporation of glucose in oral solutions might completely eliminate the requirement for intravenous fluids in the average cholera case was clearly substantiated in the symposium independently by L. Mata (Costa Rica), S. C. Pal (Calcutta), D. Barua (WHO, Geneva), W. B. Greenough, III (Dacca), T. Hendrix (Johns Hopkins), M. Field (Chicago), and M. Levine (Maryland). All but a small percentage of diarrhoeal patients can now be treated successfully solely

with orally administered electrolyte solutions. This can be accomplished, effectively and economically, by the distribution of packets containing pre-weighed amounts of glucose, NaCl, KCl and NaHCO_3 to be added to measured volumes of water in the hospital, in local treatment centres and, most importantly, in the home, even in the most rural and underdeveloped areas. In a small number of severe cases, particularly those with emesis precluding oral rehydration, intravenous fluid therapy is still required, but even some of these can be treated orally if the electrolyte solutions are administered in small but frequent increments. It is only a minor limitation that antibiotics, which have been shown to reduce the duration of bacterial shedding and of the diarrhoea, are not presently included. The impact that decentralisation of treatment might have on the ecology of the pathogens involved should be a subject of surveillance in the future. Other questions that remain to be answered are whether other energy sources can be substituted for glucose and whether different formulae are required for diarrhoea due to rotaviruses or other agents.

That certain antibiotics, administered prophylactically, can reduce the incidence of diarrhoea in travellers was established by B. Sack (Johns Hopkins University) in studies with Peace Corps volunteers. In this population, enterotoxigenic *Escherichia coli* are responsible for the majority of cases and, fortuitously at present, the majority of these strains are antibiotic sensitive. However, because of the spectres of drug resistance and alterations of normal flora leading to superinfection, participants at the conference agreed that prophylactic antibiotics, if used at all, should be restricted to short-term travellers at high risk.

The possibility of pharmacological intervention after the onset of diarrhoea was also raised and supported by experimental studies by Hendrix and I. Lönnroth (Göteborg). At the moment, two drugs, chlorpromazine and nicotinic acid, show promise in animal studies and merit further consideration for controlled study in man.

Contrary to popular belief, evidence presented by Mata indicates that rather

than malnutrition predisposing to infection, the reverse seems to be the rule—'malnutrition is determined by diarrhoea.' With newborn infants and children in particular, especially in developing areas, there is a tendency to withhold nutrients during diarrhoeal illness. Mata's studies in Central America, supported by observations of Barua, clearly indicate that when such children are adequately treated, by oral therapy, during their multiple bouts of diarrhoea, they grow better than their counterparts — the stunting which occurs during and following illness does not happen. This work has the important implication that availability of food is not the major problem in 'malnourished' children. Rather, the problem is to educate the people to provide proper treatment and normal (adequate) diet throughout periods of diarrhoeal disease. This is particularly important during the nursing period. Mata's message was also that we should be careful to differentiate between susceptibility and severity, recognising that the malnourished individuals are most frequently found in poor sanitary environments.

Epidemiologically, it was acknowledged that cholera declines when the water supply is freed of contamination with human faeces. It is not clear, however, that this is the case with other enterotoxigenic enteropathies such as *E. coli* diarrhoea, which seems to be transmitted primarily within the household and by food. The problems of developments in sanitation, which could solve the cholera problem, while recognised by the participants, were felt to be beyond the province of this conference.

Presentations by M. Gill (Harvard University) and M. Field (University of Chicago) revealed that for the second and third times (diphtheria being the first), the pathogenesis of infectious diseases is within a hair's breadth of being totally understood at the molecular level. Field, who inspired the recognition that cholera enterotoxin (cholera toxin) acts by activating host cell

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*The symposium was held at the Nordic Education Centre, Stockholm, and was excellently organised by Örjan Ouchterlony, Jan Holmgren, John Craig and Dhiman Barua. Review papers provided the foundations for workshops culminating in recommendations for the future. The complete proceedings, workshop summaries and recommendations are to be published by S. Karger AG, Basel, early in 1979.

adenylate cyclase, presented new evidence that ST (the heat-stable low molecular weight enterotoxin(s) of *E. coli*, of which one example was characterised by D. Robertson (University of Kansas) as a peptide of 47 residues) acts by activating host cell (specifically intestinal epithelium) guanylate cyclase, while Gill indicated that the A-subunit of cholera toxin catalyses primarily the ADP-ribosylation of a GTP-binding protein which, according to observations by Z. Selinger, traps adenylate cyclase in its activated state. The subsequent events, catalysed by cyclic-AMP or cyclic-GMP, which lead to the increased electrolyte and fluid secretion, are still a grey area.

S. Falkow (University of Washington, Seattle) dynamically demonstrated the application of the newly-developing recombinant DNA technology to the solution of problems of pathogenesis, with the object of creating useful new microbial strains, rather than new pathogens, with evidence for the cloning of the ST and LT (heat-labile cholera-related enterotoxin) genes of *E. coli*. He supported the observations of F. Ørskov (University of Copenhagen) that the *E. coli* strains which are selected to become enteropathogens (that is, which can effectively receive *tox* plasmids and other essential virulence factors) may be restricted to a relatively small number of serotypes which happen to be relatively good genetic recipients. Some 'non-toxicogenic' strains of *E. coli* of proven pathogenicity for man in volunteer studies may produce toxins which are active in other than the usual models, according to Levine. Other essential attributes of virulence, such as chemotaxis and adhesiveness leading to colonisation, were discussed by R. Freter (Michigan). J. Craig (New York) pointed out that cholera vibrios differ in the amount of toxin they elaborate *in vitro* under two different conditions of cultivation. The advantages and limitations of available techniques of recognising enterotoxins and their antibodies were discussed by Ö. Ouchterlony, who also presented a novel simple method of assay based upon changes in hydrophilic character of antigen-antibody complexes on plastic surfaces. The essential roles of the host cell glycolipid receptors, the G_{M1} ganglioside, elucidated by the pioneering observations of W. E. van Heyningen, were reviewed and extended by L. Svennerholm and J. Holmgren (Göteborg).

The possibilities of immunological intervention were high among the priorities of the Symposium. J. Feeley (Atlanta) extensively reviewed the scientifically controlled field studies of cholera vaccines composed of killed vibrios administered parenterally, and concluded, in accord with previous

judgment, that the benefits derived did not justify the cost of vaccination. According to Barua, in consideration of these observations, requirements for cholera vaccination were removed from WHO regulations in 1973. Nevertheless there is the possibility of improvement, as brought out by more recent studies, presented by I. Joó (Hungary) and Pal, on alum-adsorbed bacterial vaccines which seem to generate greater protection in the susceptible infants and children of endemic areas than previously tested un-adsorbed vaccines. Although cholera toxoid administered parenterally offers no protection according to the field studies summarised by N. Ohtomo (Japan) and G. Curlin (Dacca), the synergistic protective effect observed experimentally with combinations of toxin antigen and somatic antigens in studies by A.-M. Svennerholm (Göteborg) is worth further consideration. She also indicated the potential value of assaying the antibody content of milk from immunised lactating women as a reflection of the local (intestinal) secretory antibody response. N. Pierce (Johns Hopkins University) reviewed extensively his observations on the kinetics of formation of antibody-containing cells in the lamina propria in response to a variety of combinations of orally and parenterally administered toxin antigens in experimental animal models and concluded that the nature of the antigen is important and that immunological suppression may result from some regimens. J. Holmgren (Göteborg) indicated that species differences may also be important, as illustrated by his studies in the orally immunised mouse model described earlier by Fujita and Finkelstein. J. Murphy (Harvard) reviewed genetic studies in his laboratory which have resulted so far in the isolation of two hypotoxinogenic mutants of strain 569B which produce reduced amounts of subunit A and little, if any, subunit B.

That solid immunity against cholera is indeed feasible has been established conclusively by studies in volunteers as summarised by Levine. Volunteers convalescent from induced cholera were found to be virtually completely refractory to subsequent challenge with cholera vibrios of either the homologous or heterologous serotype. Therefore it is clear that the disease itself is an immunising process, although the mechanism(s) and the nature of the immunising antigen(s)—somatic antigens, enterotoxin, colonisation factors, flagella, outer membrane proteins, or a combination—are not yet known. Immunity to rechallenge after induced *E. coli* diarrhoea was markedly less effective.

In an earlier session, R. A. Finkelstein (University of Texas, Dallas) sum-

marised the laboratory production and isolation of enterotoxins; elucidated criteria for defining enterotoxins and 'enterocytotoxins'; issued a caveat against the tendency to regard all newly described toxicities as toxins; and also reported the isolation with T. Honda, of a mutant of *Vibrio cholerae* which offers promise as a live vaccine against cholera and related diarrhoeas. Discussed further in the Immunology workshop, the mutant, called 'Texas Star', is avirulent in the sensitive infant rabbit model, has shown no signs of reversion, and seems to produce only the immunogenic B (binding) portion of the cholera enterotoxin. It is hoped that the mutant will be shown to be innocuous in volunteer studies and that it will simulate infection with virulent vibrios by producing solid immunity to cholera, and potentially to other diarrhoeas as well, by means of the common toxin B antigen. As a previous hypotoxinogenic mutant, M13, isolated in our laboratory, was harmless in volunteers and induced cholera immunity, this expectation may not be unrealistic.

In the immunology workshop it was emphasised that in volunteer studies, which are an effective step toward vaccine development, the highest internationally acceptable ethical standards must be followed with regard to selection, education, and respect for the rights of volunteers.

Participants were reminded of how far we have come in a short time by the presence of one of the Indian investigators, S. N. De (Calcutta), who in the late 1950s first showed that the symptoms of cholera could be produced in laboratory models by cell-free products of the cholera vibrio. □

Ultra-cold neutrons in superfluid helium

from P. V. E. McClintock

THE first measurements of the rate at which ultra-cold neutrons are produced within a vessel of liquid helium, reported in a recent issue of *Physics Letters* (66A, 469; 1978), strongly support the earlier suggestion by Golub and Pendlebury (*Physics Letters* 62A, 337; 1977) that it might be possible to fill a neutron bottle with trapped neutrons to an unprecedentedly high density if superfluid ⁴He were used as the internal medium. The production measurements were carried out in Grenoble at the Institut Laue-Langevin by P. Ageron and W. Mampe (ILL) in collaboration with R. Golub and J. M. Pendlebury (Sussex University).

Ultra-cold neutrons (UCN) are neu-

trons of such low energy that they are totally reflected from material surfaces at all angles of incidence, so that it becomes possible to confine them inside a suitably designed container (neutron bottle), where experiments can then be conducted in a relatively leisurely fashion—compared to neutron beam experiments where the time of observation is restricted to about 1/30 s at most—to elucidate their properties. If they could be trapped at sufficient density for periods comparable with their lifetime (neutrons decay by β -emission in about 15 minutes), then it might become possible to determine certain fundamental constants, such as the electric dipole moment of the neutron for which there are various theoretical predictions but, as yet, no positive measurements. The main difficulty is that the present technology available for UCN production, exploiting the very low energy Maxwellian tail of the thermal distribution of neutrons within a nuclear reactor, leads to trapped UCN densities of only about 1 cm^{-3} , which is not really adequate for the proposed experiments.

Golub and Pendlebury's suggestion amounted to a completely different technique by which UCN might be produced: by down-scattering in liquid helium of neutrons with an initial velocity of about 450 m s^{-1} . They pointed out that, when a neutron of this particular velocity travels through a bath of superfluid ^4He , there is a finite probability that it may spontaneously create a thermal excitation (in effect, a single quantum of sound) and, in doing so, lose almost all its initial energy. In its final state it will be an ultra-cold neutron, moving at less than 5 m s^{-1} , and unable to escape from the container. Their proposed UCN source consists, therefore, of a container of superfluid ^4He whose walls are transparent to neutrons with velocity of 450 m s^{-1} , placed in a beam containing a relatively large proportion of neutrons of this velocity. Most of the neutrons will pass straight through and out the other side, but a certain number will create excitations, enter the UCN region and become trapped.

The UCN density attainable by this method clearly depends on a balance between the production rate and the loss rate. Losses of UCN from the bottle will occur inevitably through β -decay but, in addition, can also arise as the result of up-scattering by thermal excitations (the converse of the production mechanism), absorption by impurities in the ^4He , or inelastic scattering from the walls. The latter phenomenon has restricted the storage times achieved in evacuated room-temperature bottles to about 30 s, but it is expected to become much less important at the temperatures over

1 K where the helium filled bottle will be operated. The most potent neutron absorber likely to be present in the liquid is the other isotope of helium, ^3He : it will be necessary to reduce the ^3He isotopic impurity content to a level about four or five orders of magnitude below that (a few parts in 10^7) found in ordinary commercial ^4He . Fortunately, however, purities even greater than this can quite readily be achieved using the heat-flush isotopic separation method recently developed at Lancaster, and neutron absorption within the body of the liquid is not therefore expected to be a problem. Neither should up-scattering processes since, provided the bottle is operated at a temperature below 1 K, the number of excitations with the appropriate energy to initiate such events will be quite negligible. The main unknown factor has been the production rate and, although Golub and Pendlebury did their best to estimate it, the calculation was by no means straightforward and it incorporated a number of assumptions which could have been in error. Hence the importance of the work now being reported from the ILL, which constitutes the first experimental measurement of the UCN production rate in superfluid helium.

The arrangement used was extremely simple. A vessel of liquid ^4He , whose temperature could be reduced by pumping, was placed in a horizontal neutron beam. Ordinary commercial ^4He was used, and so no provision was made for storing any UCN which might be produced. Instead, a neutron guide tube was provided to lead the UCN out of the liquid helium to an external neutron detector. The guide incorporated five right-angled bends to enable neutrons of higher energy to escape through its walls and thus to ensure that only the UCN could reach the detector and be counted.

In the event, the neutron counting rate increased rapidly to about 5 s^{-1} when the temperature of the liquid was reduced below its superfluid transition temperature at 2.2 K. When a 0.5 mm thick stainless steel plate, totally opaque to UCN but partially transparent to neutrons of higher energy, was placed so as to block the guide tube, the counting rate fell to the background level of 0.5 s^{-1} ; but it returned to its former value again as soon as the plate was removed. Some 24 hours later, as the last of the liquid helium boiled away, the rate fell once more to the background level. It seems virtually certain, therefore, that the detected neutrons were UCN produced in the liquid through the mechanism proposed by Golub and Pendlebury. Furthermore, the measured production rate was found to be in remarkably good

agreement with the value which they had calculated.

This is a very encouraging result. It implies that the fullscale neutron confinement cryostat now being planned at the ILL, which will incorporate a sample of isotopically ultra-pure liquid ^4He , is likely to store UCN at densities at least sixty times larger than have been attained by conventional methods—and perhaps a thousand times larger, if the problem of wall losses can be overcome. $\square$

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Progress in Fusion Research

from a Correspondent

TOKAMAK rules. That was the essential message from the 7th International Conference on Plasma Physics and Controlled Nuclear Fusion Research held at Innsbruck from 23–30 August last. The results from the large (PLT) tokamak at Princeton (USA) were presented and discussed in some detail. In particular the scientific basis for the widely publicised claim that a temperature of 60 million degrees had been reached was carefully examined and finally accepted. The discussions highlighted the difficulties of unambiguous measurement of ion temperatures in such plasmas. Indeed it can be argued that the obsession with 'temperature' is a hangover from the Zeta days and that in these plasmas, particularly those heated by powerful neutral beams, the single crucial number is the ratio Q of the energy released in fusion reactions to the energy input. In the Princeton experiment with 1.7 MW of neutral deuterium beam power into a deuterium plasma, a neutron emission of $4 \times 10^{13}\text{ neutrons s}^{-1}$ was measured; at $\sim 3\text{ MeV}$ per reaction, one can calculate that this corresponds to $\sim 20\text{ W}$ of fusion power and hence to $Q \sim 10^{-5}$. If a tritium beam had been used, then the more favourable cross-section and larger energy release would have increased Q to $\sim 5 \times 10^{-5}$. The new tokamak TFTR at present under construction at Princeton is designed to achieve $Q > 1$ through the reduction in diffusive losses by the use of larger dimensions. In the light of the new results its chances of success look good. For a power reactor $Q \sim 20$ would be needed.

Results from other tokamak experiments around the world showed an increasing ability to reduce the contamination of the plasma by impurities. With cleaner plasmas it is possible to reach higher densities than before and to increase the ratio of toroidal plasma current to toroidal magnetic field. Thus

in the Alcator experiment a record value of the product of plasma density and containment time was reached, i.e. $\langle n\tau \rangle = 3 \times 10^{13} \text{ cm}^{-3} \text{ s}$. The plasma here was relatively cold with a temperature of ~ 8 million degrees.

Despite the success of tokamaks in confining hot plasma the difficulties of developing the concept into a practical power producing reactor are immense. One major problem is the relatively low ratio β between the plasma pressure and that of the magnetic field. The plasma produces the power while the magnetic field costs money. Thus many studies have suggested that the minimum acceptable β for a reactor is 5–10%. In present tokamaks β is typically less than 1%. Theoretical work from Culham, Lausanne and Princeton presented at the conference suggests that the maximum β in a tokamak consistent with plasma stability is only about 3%. This leaves an awkward gap and it is therefore of crucial importance to carry out definitive experiments to test the theory.

The fact that the road to the tokamak reactor is clearly a very difficult one encourages the continuation of work on other magnetic confinement approaches where the difficulties are equally daunting but different.

Thus work continues on a variety of magnetic confinement schemes including the stellarator, reversed field pinch and mirror machine. Steady progress on these lines was reported at Innsbruck but no startling breakthrough of the kind that would lead to any switch of emphasis away from the tokamak. A dark horse in the competition is the Elmo Bumpy Torus (EBT) experiment at Oak Ridge. In this experiment a steady state toroidal plasma is produced by high power (60 kW) microwave heating with an ion temperature of about 2 million degrees. The practicability of this approach has recently been greatly increased by technical developments in the generation of high power millimetre microwaves. A reactor design based on the EBT principle was presented at Innsbruck. A microwave power of the order of 100 MW at $\sim 100 \text{ GHz}$ is envisaged.

The major alternative to magnetic confinement is the inertial approach in which a tiny pellet is compressed to densities of $\sim 10^{25} \text{ particles cm}^{-3}$, heated to thermonuclear temperatures and holds together under inertial forces for long enough (10^{-11} s) to give an adequate yield of fusion energy. Presentations on this subject at Innsbruck were relatively muted, the major groups being mainly pre-occupied with getting the necessary multi-terawatt driver systems (lasers, ion beams and electron beams) to work. Thus the essential

feature of compression to very high densities has yet to be experimentally demonstrated. Most existing experiments are done with so called exploding pusher targets. In these high pressure (100 atm) deuterium and tritium gas is compressed by the explosion of the surrounding glass microsphere. Experiments of this type around the world with CO_2 and Nd glass lasers (including one at the Rutherford Laboratory), have produced a great deal of information, diagnostic experience and Q values of $\sim 10^{-5}$. Experiments aimed at $Q \sim 1$ using the Shiva laser at the Lawrence Livermore Laboratory (Nd glass, 26 TW) and the Antares laser at Los

Alamos (CO_2 , 100 TW) are due to begin soon. The problems of converting these inertial confinement systems into fusion reactors with the necessary high repetition rate and adequate containment of the successive small explosions are very great. The Livermore group propose to surround the exploding targets with a 100 ton, 1 m thick falling curtain of liquid lithium to absorb the neutrons and reduce radiation damage to the external structure.

Certainly all the concepts presented at Innsbruck lead to very complex and difficult reactors. The assessment and mastery of this complexity will be one of the major tasks for the coming years. $\square$

Zr₃Al creeps in a petty pace

from Robert W. Cahn

IN 1956, A. H. Cottrell predicted that metallic uranium, while undergoing steady fission, would lose most of its resistance to creep under stress, and he initiated a very simple experiment with a loaded uranium spring inside a reactor. His hypothesis of enhanced irradiation-creep was promptly confirmed and this had immediate consequences for the design of the British nuclear reactors of that time.

Cottrell's crucial hunch has been followed up in the intervening years by a large body of experiment and theory. Not only a fuel but also its container is subject to this form of creep, and it is a matter of special concern when a 'cladding material' is weakened, for the container has the function of retaining fission products both solid and gaseous; the latter exert a pressure which must be resisted by the container. After much experiment as well as very sophisticated theoretical physics during the past few years, the mechanisms both of irradiation-creep and of the related process of void-swelling (when minute cavities form in the container during reactor service) are now understood in outline. Neutron irradiation produces equal numbers of vacancies and interstitials, some of which recombine quickly; the rest migrate to and become absorbed at 'sinks', of which dislocations are the most important. The crucial point is that a dislocation is a 'biased sink'—that is to say, it has a slightly greater tendency to absorb interstitials than vacancies (the bias is typically around 2%); this bias is explicable in terms of differing elastic interactions of the dislocation with the two types of point defect. The excess of vacancies so generated eventually condenses to form the minute aggregates called voids (whose existence was first reported in *Nature* in 1966). Furthermore, an external stress can increase the bias and thus

accelerate the climb of dislocations which must result from a net flux of interstitials to dislocations. That accelerated climb in turn is apt to produce accelerated creep under the action of the external stress, since under certain circumstances, the climb rate is rate-determining for creep.

The recognition of the central importance of point defect kinetics in irradiated alloys has opened the way for a new kind of alloy design—the attempt to predict what kind of alloy might be least susceptible to enhanced creep and void-swelling. The matter is of special interest in fast reactors and in water reactors; the latter all use container materials based on metallic zirconium, which combines an appropriately low neutron capture cross section with good corrosion resistance in hot water under irradiation. A determined attempt is now under way in Canada to improve on the 'zircalloys' in current use, and the project is based on a most ingenious hypothesis—the postulated influence of atomic order in an alloy, Zr_3Al , in reducing the net flux of interstitials to dislocations and, thus, in reducing liability to irradiation-creep.

It is well established from comparative studies of successful and unsuccessful industrial innovations that the most important single factor in ensuring success is a resolute and energetic 'product champion'. The champion of Zr_3Al is E. M. Schulson, a metallurgist on the staff of Chalk River Nuclear Laboratories in Canada. He argued in 1972 that this alloy had the virtues of low neutron absorption and promising mechanical properties, and further suggested that irradiation-creep might be reduced. The basic postulate here is that ordering generally reduces the rate of vacancy migration in an alloy (there is an often cited study of beta-brass, in 1956, which first estab-

lished this point); at any given temperature, a slowing down of vacancy migration gives more time for recombination of vacancies and interstitials and this reduces the net flow of interstitials to biased sinks, and thereby reduces irradiation-creep rate under a given stress. In pursuit of this hypothesis, Schulson has orchestrated a large range of studies, most of which have come to publication during the past 1½ years: it now looks as though his ingenious hypothesis is justified, though a full study of the irradiation-creep itself is yet to appear.

The hypothesis itself is outlined in a paper by Schulson in *J. Nucl. Mat.* **66**, 322; 1977. Here he also reports that stress-relaxation of Zr_3Al under neutron or 1 MeV electron irradiation at 570 K (a realistic reactor temperature) is a factor of three or more lower than that for any zirconium-based alloy studied to date. This striking experiment is to be fully described in a conference report to be published by ASTM. Zr_3Al is ordered (Cu_3Au type, Ll_2) up to its peritectoid transformation at 1,265 K, but can be progressively disordered by radiation damage. Several studies have sought to establish whether, at temperatures of practical interest, radiation-disordering goes far enough to nullify the advantage which forms the basis of Schulson's hypothesis. This is a matter of balance between radiation damage and spontaneous reordering, and this balance depends on the rate of damage and the temperature. Accelerated damage by ion bombardment (Howe & Rainville *J. Nucl. Mat.* **68**, 215; 1977) or 1 MeV electron bombardment (Carpenter & Schulson *J. Nucl. Mat.* **73**, 180; 1978) showed that at realistic service temperatures, large doses leading to several displacements per atom were required to produce substantial disorder above 575 K. Neutron bombardment in reactors would produce less damage and therefore less disorder than either ions or 1 MeV electrons. Further studies (Rosinger *J. Nucl. Mat.* **66**, 193; 1977; Schulson, *Acta Met.* **26**, 1189; 1978) have established that neutron irradiation strengthens Zr_3Al at all temperatures, and another (Schulson & Roy *J. Nucl. Mat.* **71**, 124; 1977) has elicited the remarkable fact that irradiation actually reduces the notch-sensitivity of the alloy (which is in any case not severe) and, at room temperature, the notched tensile strength is even enhanced by irradiation. This behaviour is quite at variance with the normal effect of irradiation on notch-brittleness, which is pronounced, for instance, in ferritic steels. Another study (Schulson *Met. Trans.* **9A**, 527; 1978) established that the fracture morphology of Zr_3Al indicates a ductile fracture mechanism.

These various experimental studies have now been supplemented by a thorough theoretical investigation of the kinetics of point defect annihilation and irradiation creep for a number of idealised imaginary alloys with various types of atomic order (Schulson, Swanson & MacEwen *Phil. Mag.* **A37**, 575; 1978). This very thorough analysis is based on certain assumptions about the change of migration energy for vacancies as a result of ordering and the further assumption that sink strengths are not affected by ordering. Numerical results are obtained for vacancy concentration as a function of temperature, for net interstitial flux to dislocations for various assumed vacancy migration energies and, finally, for the factor of reduction of dislocation climb rate due to ordering. In the temperature range of interest, for the

idealised Ll_2 alloy, the climb rate (and therefore the irradiation-creep rate) is shown to be reduced by 30–70%, depending on dislocation density and whether simple or super-dislocations are present. This paper marks a milestone in the application of exact atomistic theory to the prediction of irradiation behaviour.

Zr_3Al may turn out to behave even better than theory indicates, because preliminary electron microscopy indicates that the alloy, after irradiation, has a smaller than normal density of dislocation loops. Some unidentified healing mechanism, it seems, may render the alloy even less liable to the processes that enhance irradiation-creep than is predicted by theory.

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Unfolding Troponin-C

from Jonathan Seymour

TROPONIN-C (TN-C) is one of the most interesting proteins in muscle, because it lies at the kernel of the mechanism for controlling contraction and relaxation. When the concentration of Ca^{2+} around the thick and thin filaments of muscle is high, TN-C binds Ca^{2+} and contraction takes place. In contrast, when the Ca^{2+} concentration is low, TN-C loses its bound Ca^{2+} and contraction is inhibited and the muscle relaxes. This inhibition of contraction is produced by tropomyosin and the troponin complex, both of which are situated on the muscle thin filaments. The troponin complex consists of three components: TN-I, which exerts the inhibitory action; TN-T, which binds the complex to tropomyosin and TN-C, which is responsible for binding Ca^{2+} . Recent experiments have revealed how the structure and functions of TN-C are related.

TN-C has two binding sites which can bind either Ca^{2+} or Mg^{2+} . At low concentrations of Mg^{2+} , these Ca^{2+} - Mg^{2+} sites show a high affinity for Ca^{2+} . Also TN-C has two further sites that can bind Ca^{2+} only. These Ca^{2+} -specific sites show a lower affinity for Ca^{2+} than the Ca^{2+} - Mg^{2+} sites, in the absence of Mg^{2+} . The application of such techniques as proton magnetic resonance, circular dichroism and a measurement of tyrosine fluorescence have revealed the effects of Ca^{2+} binding at the different sites of TN-C. When Ca^{2+} binds to the Ca^{2+} - Mg^{2+} sites, the backbone of the polypeptide chain refolds, generating new lengths of α -helix. This large re-organisation of the molecule results in TN-C adopting a more stable conformation. Similar, but not identical, changes occur when Mg^{2+} is bound to the Ca^{2+} - Mg^{2+} sites. Further binding of Ca^{2+} at the Ca^{2+} -

specific sites results in smaller changes occurring, but there is some evidence for an accompanying increase in α -helix.

The primary sequence of TN-C reveals four regions (I to IV) of the polypeptide chain that are homologous with the Ca^{2+} binding sites of parvalbumin. Parvalbumin is a Ca^{2+} binding protein whose three dimensional structure has been solved, to an atomic resolution, by X-ray crystallography. These Ca^{2+} binding sites consist of a bend in the polypeptide chain, rich in acidic side chains, and flanked on both sides by α -helical regions. The immediate question arises, which regions of the sequence (I to IV) correspond to Ca^{2+} - Mg^{2+} sites and which to Ca^{2+} -specific sites? Detailed analyses of the sequences in the four regions led to contradictory results. Further progress was made by attaching probes to one cysteine residue in region III of the molecule. Using fluorescence measurements and E. S. R. techniques, it was established by Potter *et al.* (*J. of Biol. Chem.*, **251**, 7551; 1976) that region III corresponded to a Ca^{2+} - Mg^{2+} site.

Recently, the nature of all four regions has been unambiguously resolved by studying the Ca^{2+} binding properties of fragments of TN-C. Leavis *et al.* (*J. of Biol. Chem.*, **253**, 5452; 1978) used trypsin, thrombin and cyanogen bromide to cleave TN-C at different points along its polypeptide chain. The resulting fragments contained one or more of the four binding regions (I to IV). It was shown that regions III and IV corresponded to the Ca^{2+} - Mg^{2+} high affinity sites, in agreement with the earlier assignment of region III. It follows immediately that regions I and II correspond to the Ca^{2+} -specific sites. In addition two regions

of TN-C were identified that bound to TN-I. This is in agreement with similar work done on TN-C fragments by Weeks and Perry (*Biochem. J.*, **173**, 449; 1978) and also with earlier work done on TN-I.

At the same time the work of Leavis *et al.* revealed a limitation of looking at fragments of TN-C. It was found that fragments containing the two Ca^{2+} - Mg^{2+} sites, regions III and IV, had a strong affinity for Ca^{2+} and could also bind Mg^{2+} . Further cleaving the polypeptide, so that regions III and IV were on separate fragments, resulted in the isolated Ca^{2+} - Mg^{2+} binding sites changing their characteristics. Now they could only bind Ca^{2+} weakly and failed to bind any Mg^{2+} at all. This illustrates the importance of tertiary structure (relationships between different regions of the same molecule) for defining the properties of the binding sites. Indeed, the Ca^{2+} -binding properties of TN-C are related to its quaternary structure (relations to surrounding protein molecules), because when TN-C binds to TN-I, its affinity for Ca^{2+} increases by an order of magnitude.

In order to obtain a better understanding of how Ca^{2+} binding to TN-C releases the inhibitory effect of the troponin complex and tropomyosin, it is crucial to know which Ca^{2+} binding sites on TN-C are the regulatory ones. The bulk of the evidence suggests that it is the Ca^{2+} -specific sites that regulate contraction. Work with myofibril preparations indicate that the Ca^{2+} concentration at which their ATPase rate is activated is independent of the concentration of Mg^{2+} . Hence, only the Ca^{2+} -specific sites are involved in regulation; but work on the tension development in muscle suggests that raising the concentration of Mg^{2+} increases the Ca^{2+} concentration required for the muscle to be activated. This possibly implicates the Ca^{2+} - Mg^{2+} sites in regulation.

The solution of this dilemma would be an important advance in understanding how TN-C regulates contraction. A crucial piece of data relevant to this question is the concentration of Mg^{2+} in muscle. This is not accurately known, but it is believed to be in the range 0.1 to 5mM. This knowledge would enable the metal ions bound to TN-C to be identified in contracting and relaxed muscle. When the Ca^{2+} concentration is high and the muscle is contracting, all four sites on TN-C bind Ca^{2+} . However in relaxed muscle when the Ca^{2+} concentration is low, the Ca^{2+} -specific sites are free of Ca^{2+} , while the Ca^{2+} - Mg^{2+} sites could be binding Ca^{2+} or Mg^{2+} , depending upon the concentration of Mg^{2+} . There is also much more to learn about the relationships between TN-C and its

neighbours TN-I and TN-T. For it is these changing relationships, as TN-C binds and releases Ca^{2+} , that release and trigger the inhibitory action of troponin and tropomyosin.

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Nuclear resonance & synchrotron radiation

from G. V. Marr

THE electromagnetic radiation (synchrotron radiation) emitted by high energy electrons when they are constrained by magnetic fields to move in circular orbits in an electron synchrotron or storage ring is now well known and much used as an intense source of ultraviolet and X-ray photons. Because the electrons are injected as localised bunches into the synchrotron and are accelerated to speeds approaching the speed of light, the synchrotron radiation emitted is highly polarised and concentrated into tightly collimated beams forward-directed along tangents to the instantaneous electron orbit. The radiation emitted is characterised by the electron energy and the bending radius of the orbit so that its spatial and energy distribution is calculable. It appears as a continuum of radiation extending from the infra-red to the X-ray region with a peak in the XUV or X-ray region. It also possesses a pulsed-time structure determined by the orbiting frequency of the electron bunches. There are now more than 20 laboratories throughout the world, 7 of which are in Western Europe, where facilities exist for the exploitation of synchrotron radiation and research programmes are in operation to study problems in atomic, molecular, surface and solid state physics, in chemistry, and in the structural properties of solids and biological samples. In addition, X-ray microscopy, lithography, the calibration of light sources and detectors, etc., are applications of synchrotron radiation.

New applications are continuously appearing and an interesting extension of these activities to the observation of nuclear resonance excitation has been reported by R. L. Cohen *et al.* (*Phys. Rev. Letters* **41**, 381; 1978). The interest is not so much that new data on the nucleus has been revealed, indeed the observations are of the well known 14 keV level of ^{57}Fe , but that the apparatus developed enables the resonance to be detected by the observation of a weak flux of conversion electrons emitted by the decay of the nuclear state in the presence of a much more intense electron flux from non-

nuclear electronic processes.

The method makes use of the fact that the University of Stanford storage ring can provide pulses of synchrotron radiation 0.3 ns wide every 780 ns. With a long lifetime (100 ns) against decay of the nuclear level it has been possible to gate the detector system so as to collect data 100 ns following the incidence of the synchrotron radiation pulses, thereby allowing photoelectrons, Compton electrons and long lived secondary electrons to dissipate before the detector is activated.

The synchrotron radiation was dispersed by a crystal X-ray monochromator to provide a band width of 3 eV photon energy centred about the 14 keV excitation energy. This radiation fell onto a ^{57}Fe enriched iron foil and the emitted electrons entered a continuous semi-conducting dynode electron multiplier which was divided into two stages with a gated grid between the two sections. The grid was gated "off" during the prompt photoelectron pulse and so removed the main cause of afterpulsing electrons which would be otherwise indistinguishable from the nuclear conversion electrons. Very low energy electrons having flight times of as long as 100 ns were removed at the electron multiplier entrance.

Under these conditions, the crystal monochromator was scanned over the energy of the ^{57}Fe nuclear resonance and the counting rate plotted as a function of X-ray energy. The width of the resonance is negligible (5×10^{-7} eV) compared with the band width of the monochromator so that the scan actually profiled the transmission function of the monochromator and the width and shape of the response reflect the monochromator output. Nevertheless the resonance was observed to occur at exactly the anticipated energy and was of the correct intensity. The authors also point out that their detector system can be used with advantage to provide a study of the Bragg-scattering profile of crystal monochromators in synchrotron radiation beam lines, thus allowing maladjustment of the monochromator or defining slits to be corrected *in situ*.

New synchrotron radiation sources are being designed specifically to make use of the synchrotron radiation rather than to provide high energy particles. In particular, the new 2 GeV storage ring under construction at the Daresbury Laboratory in the U.K. will be used to study the time structure of the radiation. Looking further ahead, the European Science Foundation is currently considering the design requirements for a European Synchrotron Radiation Facility for the 1980s.

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review article

Fibronectins—adhesive glycoproteins of cell surface and blood

Kenneth M. Yamada & Kenneth Olden*

A recently characterised class of adhesive, high molecular weight glycoproteins is present on the surfaces of cells, in connective tissue matrices, and in extracellular fluids. These proteins may have important roles in cellular adhesion, malignant transformation, reticuloendothelial system function, and embryonic differentiation.

RECENT attempts to unravel the molecular mechanisms of cellular adhesion, malignancy, hemostasis, host defense by the reticuloendothelial system, and connective tissue structure have implicated glycoproteins with subunit molecular weights of 200,000–250,000. These proteins are now thought to be closely related, and are termed fibronectins (from the Latin *fibra*, fibre, and *nectere*, to bind, tie)¹. We shall review the discovery, structure, and possible biological roles of these glycoproteins (for related reviews, see refs 2–6).

Discovery and nomenclature

In 1948, Morrison *et al.*⁷ isolated a partially purified fraction of human plasma which they termed 'cold-insoluble globulin'. Other workers independently described the various proteins or factors listed in Table 1, which were named according to sources or biological activities (Table 2). Recent evidence indicates that all of these proteins are closely related, and that they are probably only one or two specific proteins—cell surface (cellular) fibronectin, and plasma fibronectin. Cell surface fibronectin is a major constituent of the cell surface of many cultured cells, and was discovered when cell surface proteins or carbohydrates were labelled radioisotopically or immunologically (Tables 1 and 3). This glycoprotein is also known as LETS protein¹⁹ or CSP²⁰, and has an apparent subunit molecular weight of between 200,000 and 250,000 by SDS–polyacrylamide gel electrophoresis^{2–6}. Plasma fibronectin is a dimeric glycoprotein with subunit polypeptides of 200,000–220,000 that circulates in vertebrate blood^{7,23–26}. Although also known as cold-insoluble globulin, purified plasma fibronectin is relatively soluble in the cold unless it is complexed with fibrin and fibrinogen^{7,23–25,27,28}.

Antisera raised against the plasma or cell surface forms of fibronectin readily cross-react, and antibodies specific for only one form have not yet been described^{13,27,29–31}. Monospecific anti-fibronectin antisera against either form specifically immunoprecipitate only fibronectin from cell homogenates^{32–34}. Current information summarised below suggests that although the two forms of fibronectin are very similar, they are probably not identical. Whether they are products of different genes, or of one gene and are modified post-transcriptionally, is not yet known.

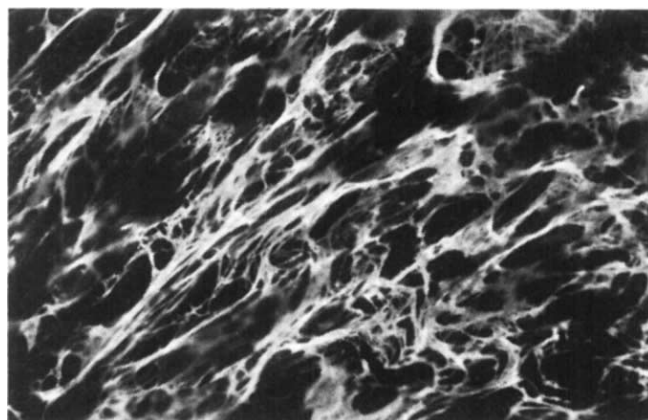
Quantities and location

Cell surface fibronectin constitutes 1–3% of total cellular protein of embryonic or adult fibroblasts cultured *in vitro*³⁵. A few

permanent cell lines also display large amounts of fibronectin, but many other permanent fibroblastic lines have much smaller quantities that are detectable only by cell-surface labelling techniques³⁵. Cell surface fibronectin has also been found on astroglial cells³⁰ and on certain cultured epithelial cells^{36,37}, including blood vessel endothelial cells^{38,39,114}.

Cell surface fibronectin is located in striking fibrillar arrays on the surface of many cultured cells, as determined by immunolocalisation studies with anti-fibronectin antibodies (Fig. 1)^{3,6,29,30,32–46}. Cell surface fibronectin extends between cells, and is also found between cells and the tissue culture substratum^{3,29,32–34}. This localisation is consistent with a role in cell–cell and cell–substratum adhesiveness. In densely confluent cultures, cellular fibronectin forms a complex, interconnected extracellular matrix that may contain collagen or elastin^{3,10,12,29,32–46}. Electron microscopic localisation studies confirm that most of the fibronectin is present in extracellular fibrils and aggregates, with only small amounts present diffusely distributed along the outer surface of the plasma membrane of cells^{37,43–45,47}. Most cellular fibronectin can be detached and separated from plasma membranes centrifuged in sucrose gradients^{48,49}, and as much as half can be released from living cells incubated in 1M urea^{50,51}. Most of the protein therefore seems to be loosely associated with the membrane. In addition,

Fig. 1 Cell surface fibronectin on chick fibroblasts *in vitro*. A confluent culture was fixed and stained with affinity purified anti-fibronectin followed by fluorescein-labelled anti-immunoglobulin. $\times 520$.



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large amounts of cellular fibronectin are also continuously secreted or sloughed from cells into culture media^{10,20,30,37,53-58}

Fluorescently labelled antibodies have also been used to determine the locations of the fibronectins in embryonic and adult tissues. Fibronectin is present as extracellular fibrils in connective tissue, in primitive mesenchyme, and in many basement membranes^{59,60}. During embryonic development, fibronectin is first detectable on the cells of the blastula inner cell mass⁶¹. Later in development, fibronectin is lost as mesenchymal cells differentiate into muscle, cartilage, and renal tubular epithelium^{59,62}. It is not present in hepatocytes^{59,60}, in contrast to many other plasma proteins. Fibronectin is also present in amniotic and cerebrospinal fluids^{37,63,64}, and on platelets²³. Since anti-cell surface and anti-plasma fibronectins cross-react, it is important to note that most of these immunological studies do not indicate whether the fibronectins at each of these sites are the cellular or plasma form.

Plasma fibronectin (cold-insoluble globulin) is present in human plasma at 0.3 mg ml⁻¹ (ref. 23). As it binds to fibrin, its concentrations are lower in serum²³. Plasma fibronectin also binds to heparin, and together with fibrinogen will also form a cryoprecipitate⁶⁵. Plasma fibronectin can be covalently cross-linked to fibrinogen or to itself by plasma transglutaminase (activated factor XIII)^{25,26}. Factor XIII can also covalently crosslink cellular fibronectin to itself^{66,67}.

Concentrations of fibronectin in blood are decreased after extensive trauma, in the disseminated intravascular coagulation syndrome, and after reticuloendothelial system blockade following injection of colloids in animals⁶⁸⁻⁷². Levels of the protein are often elevated in cirrhosis and the cholestasis of pregnancy, possibly because of defective hepatic removal⁷³.

Decrease after malignant transformation

A major source of interest in fibronectin is the finding that the amount of cell surface fibronectin is usually decreased after cell lines are transformed by oncogenic viruses or carcinogens, and on cultured tumour cells (Table 3). However, there are exceptions to this generalisation, particularly among spontaneously transformed cell lines (Table 3).

The tumorigenicity (capacity to form benign or malignant tumours after injection into immunosuppressed or immunocompatible animals) of cell lines generally correlates with decreases in cell surface fibronectin^{29,45,99,100}, although a recent study of closely related transformed cells grown in semi-solid medium has cast doubt on this relationship (P. H. Gallimore, J. K. McDougall, and L. B. Chen, personal communication; see also ref. 95). Instead, decreases in this protein may correlate more with the propensity of cells to metastasise⁴⁵.

The decreases in cell-associated fibronectin after malignant transformation are 5- to 7-fold in chick fibroblasts and more than 10-fold in human fibroblasts and glial cells^{30,52,55}. However,

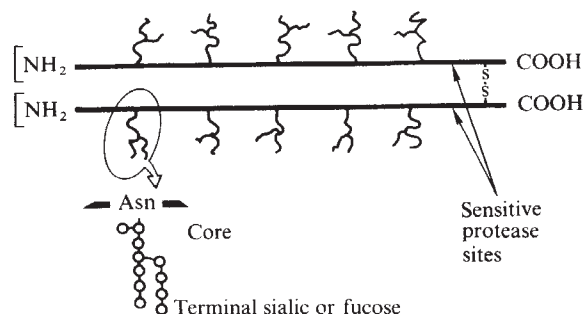


Fig. 2 Current model of fibronectin structure. Note elongated shape and distribution of inter-subunit disulphide bonds, adjacent sites that are highly sensitive to the proteases plasmin and trypsin, and the blocked amino termini. The oligosaccharides consist of a core of *N*-acetylglucosamine and mannose linked to asparagine residues with distal galactose residues that may be linked to terminal sialic acid or fucose residues.

Table 1 Nomenclature of glycoprotein factors

Cold insoluble globulin ⁷
Anti-gelatin factor ⁸
Microfibrillar protein ^{9,10*}
Opsonic protein ¹¹
Fibroblast surface antigen (SFA) ^{12,13*}
Galactoprotein a ^{14,15*}
Cell attachment factor (CAF, c-CAP) ¹⁶⁻¹⁸
Large, external, transformation-sensitive (LETS) protein ^{19*}
Cell surface protein (CSP) ^{20*}
Zeta (Z) ^{21*}
Cell spreading factor ²²
Fibronectin ¹

Asterisks indicate cellular fibronectin.

decreases of more than 10-fold in cell surface fibronectin also accompany the establishment of several permanent rodent cell lines and these decreases are not accompanied by the acquisition of tumorigenicity³⁵. The potent tumour promoter phorbol myristate acetate also causes a reversible loss of fibronectin¹⁰¹.

It is obviously important to resolve whether there is any direct causal relationship between decreases below specific quantities of cell surface fibronectin and the propensity of cells to form tumours or to metastasise.

Functions of the fibronectins in adhesion

Fibronectins are thought to be involved in a wide variety of cellular activities (Table 2). The simplest unifying interpretation of these findings is that the fibronectins function as adhesive proteins that bind cells to other cells or to substrata (Fig. 3). This conclusion is based on experiments *in vitro* utilising model systems of cellular adhesion, in which purified fibronectin is found to increase the adhesion of cells to cells or to the substrata on which they are grown.

The first evidence suggesting that cellular fibronectin may play a part in cell-cell adhesiveness was the finding that fibronectin purified from cell surfaces behaves like a lectin and readily agglutinates fixed sheep erythrocytes in concentrations as low as 1 µg protein per ml (refs 50, 58). Purified cellular fibronectin binds directly to fixed erythrocytes and can be recovered intact⁵⁰. Proteases and chelating agents destroy the agglutinin activity; these agents are known to disrupt cell-cell adhesion⁵⁰. Purified cell surface fibronectin can also increase the aggregation of living dissociated chick embryo and baby hamster kidney (BHK) cells¹⁰².

Purified cell surface fibronectin can also mediate the attachment of dissociated cells to collagen^{17,102}. Treatment with cellular fibronectin also increases the extent or attachment or the resistance to detachment of several transformed cell lines to plastic tissue culture substrata^{56,103,104} (see also ref. 105).

Serum factors are required for the attachment of certain cells to collagen-coated dishes^{16,17,106,107}, or for cells to attach and spread on tissue culture dishes¹⁰⁸⁻¹¹¹. These cell attachment and cell spreading factors are now thought to be serum fibronectin (cold insoluble globulin)¹¹¹⁻¹¹³. Such serum factors can bind directly to collagen or tissue culture dishes in the absence of cells^{16,17,106,108-110}. The fibronectin-mediated attachment of cells to collagen or spreading on to plastic substrata requires divalent cations such as calcium and the expenditure of cellular metabolic energy^{16-18,106,108}. Certain cells with high levels of cell surface fibronectin do not require serum fibronectin for spreading⁵. These cells may use cell surface fibronectin, since cellular fibronectin is equally active in promoting cell spreading and attachment to collagen¹⁷².

Both plasma and cell surface fibronectins can be purified by affinity chromatography on columns to which denatured collagen has been covalently coupled^{102,107,115-117,123}. Denatured collagens types I, II, and III are substantially more effective in binding plasma fibronectin than native collagens^{116,117}. The specific sites of interaction of plasma fibronectin with denatured collagen consist of a strong binding site at residues 643-819 of

the collagen $\alpha 1$ chain, plus several other weaker sites^{117,118}. Type III is the most effective native collagen in binding plasma fibronectin^{116,117}. The physiological significance of these differences in binding is not yet known.

Plasma fibronectin may play an important part in the removal of collagenous and other debris from blood after injury via the sessile macrophages of the reticuloendothelial system. An extensive series of experiments had identified an 'opsonic protein' in plasma that was involved in reticuloendothelial system clearance of injected gelatin-coated colloidal particles from blood^{11,69-71,119-121}. Other independent studies had identified an 'anti-gelatin factor' in serum that would bind to gelatin, and that was required for macrophage ingestion of denatured collagen^{8,122,123}. Both the opsonic protein and anti-gelatin factor have been purified recently, and both seem to be identical to plasma fibronectin (cold-insoluble globulin)¹²⁴⁻¹²⁶.

Are all of these biological activities of the fibronectins shared by plasma and cell surface forms? Recent experiments suggest that although both proteins are equally active in promoting cell spreading¹¹³ and attachment to collagen, cellular fibronectin is more effective in haemagglutination and in restoring adhesiveness and normal morphology to transformed fibroblasts (see below). Nevertheless, it is clear that both fibronectins display adhesivity.

Reconstitution and inactivation experiments

A potentially more general approach to determining the functions of the cell surface form of fibronectin is to reconstitute it experimentally on the surface of cells missing this protein, that is, on to transformed cells. These experiments can help to identify which of the alterations in cell morphology or behaviour occurring after cells are transformed could be due to the loss of this protein.

In 14 transformed fibroblastic cell lines examined to date, purified cellular fibronectin partially restores a more normal fibroblastic appearance to the cells^{44,56,102,103,127}. The effects include restoration of the flattened cell shape, elongated processes, smooth cell surface, and prominent intracellular bundles of microfilaments containing actin and myosin that are characteristic of normal fibroblasts *in vitro*^{44,56,102-104,127}. In these experiments, amounts of cell surface fibronectin equal to those present on freshly explanted fibroblasts can be restored to the transformed cells¹⁰³, although the reconstituted fibronectin may not bind as efficiently as originally¹⁰⁷. The reconstituted fibronectin seems to be present in normal sites as evaluated by immunofluorescence^{34,56} and by its capacity to undergo normal antibody-induced redistribution after incubation with u)fibronectin antibodies³⁴ (see below).

Table 2 Biological activities of the fibronectins

Cell-cell aggregation
Fixed erythrocytes
Live cells
Cell-substratum adhesiveness
Collagen substratum*
Plastic substratum
Cell spreading*
Reversion of transformed phenotype
Cell alignment and overlapping
Fibroblastic morphology
Cell surface morphology
Microfilament bundle organisation
Increased cell motility
Reticuloendothelial system clearance of colloids
Liver
Isolated macrophages
Specific binding to macromolecules
Collagen (denatured and type III native)*
Fibrin and fibrinogen
Heparin

Asterisks indicate activity known to be shared by both plasma and cellular fibronectins.

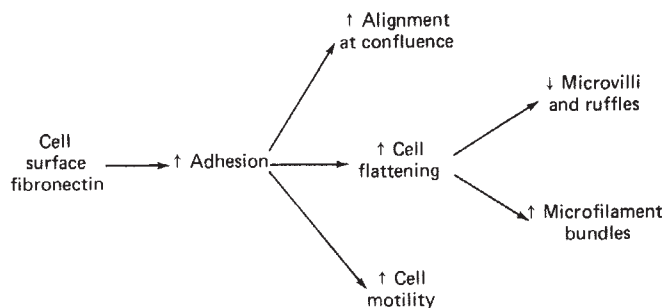


Fig. 3 Suggested relationship of cellular fibronectin to several biological activities (see refs 3, 102, 171 for discussion of evidence for this hypothetical scheme). The central activity of fibronectin would be to increase cell-cell and cell-substratum adhesion.

Besides affecting morphology, fibronectin treatment can restore a more normal social behaviour *in vitro*. For example, transformed fibroblastic cells tend to grow in relatively random patterns with cell bodies criss-crossing over one another, whereas normal fibroblasts tend to align in parallel arrays of cells. Fibronectin-reconstituted transformed cells show a restoration of this social alignment in approximately half of the cell lines tested^{56,102,103}. This defect in social behaviour is a common characteristic of cells transformed by oncogenic viruses and may result from a decrease in cell adhesion or to changes in specific cell recognition events such as contact inhibition of movement¹²⁸⁻¹³⁰. Another factor involved in maintaining cell alignment is released from cells by 0.2 M urea treatment¹³¹; however, this factor and fibronectin are clearly different molecules^{20,173}.

Fibronectin-treated cells also migrate more rapidly, both as single cells or as masses of cells migrating out from cell aggregates^{102,132,133}. These effects could be the result of establishing more effective cell-to-substratum traction¹³², or of a more asymmetric cell shape that promotes directed cell movements^{35,56,103,133}.

A complementary approach to investigating the biological activity of fibronectin is to incapacitate it with proteases or anti-fibronectin antibodies. Treatment of chick fibroblasts with purified antibody results in a rapid alteration in cell shape to a rounded morphology resembling that of many transformed cells that are deficient in cellular fibronectin³⁴. Cells treated with proteases that do not remove fibronectin tend to aggregate, whereas its removal results in cell retraction without such aggregation¹³⁴. These results are also consistent with a role for fibronectin in cell adhesion.

Cellular fibronectin does not appear to directly control cell growth rates, nutrient transport, or cyclic AMP levels. For example, these changes could not be reverted to normal after fibronectin treatment of transformed cells^{44,56,103,135}.

Protease experiments also provide additional evidence against a direct role for fibronectin in growth regulation. For example, treatment with papain or thermolysin removes all detectable fibronectin from the cell surface, but does not activate growth of chick fibroblasts¹³⁴. Conversely, thrombin activates cell division without removing cellular fibronectin^{136,137}. Insulin treatment can also stimulate mitosis without decreasing fibronectin⁷⁸.

Structure and composition

A current model for the structure of the fibronectins is shown in Fig. 2. Both plasma and cell surface fibronectins are probably elongated rather than globular proteins^{24,26,138} (contrast ref. 6). Plasma fibronectin is a dimer of two polypeptides of 220,000 each²⁴⁻²⁶. Its subunits may not be identical, since analysis of plasma fibronectin after reducing disulphide bonds reveals a closely spaced doublet band^{24,26}. Cell surface fibronectin exists both as disulphide-bonded dimers and multimers^{31,139,140}. The interchain disulphide bridge(s) of both forms of fibronectin are confined to one end of the molecule, probably the carboxyl

Table 3 Effects of transformation on cell surface fibronectin

Species and cell type	Agent		Refs
	Fibronectin decreased	Not decreased	
Chicken			
Embryo fibroblast	Rous sarcoma virus (8), B77 avian sarcoma virus, avian erythroblastosis virus	—	15, 40, 49, 53, 55, 74–80
Hamster			
Embryo fibroblast	Carcinogen (3)	—	81
Embryo kidney	Adenovirus	—	29
Nil	Hamster sarcoma virus, polyoma virus	—	8, 82–84
	Spontaneous (9)	Spontaneous	18
BHK	Polyoma	—	82, 85, 86
Tumour explant	Spontaneous	—	29
Human			
Embryo fibroblast	SV40	—	52
Tumour explants	Spontaneous (13)	—	29, 30, 52, 87
Mouse			
Embryo cells	—	Spontaneous	88
3T3	SV40, polyoma virus, Moloney sarcoma virus	SV40 cell mutant, polyoma virus	89–91
C3H/10T 1/2	Carcinogen	Carcinogen (3)	92
Explants	Spontaneous (3), [L929]	Spontaneous (2), [L929]	29, 87, 93–95
Tumour explants	Spontaneous (11), carcinogen	—	29, 87
Rat			
Embryo fibroblast	Adenovirus (4), Rous sarcoma virus	Adenovirus	29
F111 fibroblast	Carcinogen (2)	Polyoma virus	96
NRK	Rous sarcoma virus, B77	—	74, 97
Embryo myoblast	Adenovirus	—	29
L8 myoblast	Rous sarcoma virus (3), B77	—	98
Tumour explants	Spontaneous (2)	—	29
Totals	77	12	

Numbers in parentheses indicate the number of different strains or independent variants of each agent examined, if greater than 1.

terminus^{26,102,107,141,142}. This asymmetric location may serve to increase the asymmetry of the molecule. Reduction of disulphide bonds inhibits the effects of cellular fibronectin on cell–substratum adhesion¹⁴³. The amino termini of both forms are blocked^{24,26,51}, and their amino acid and carbohydrate compositions are very similar^{24,51,144,145}.

Information about the secondary and tertiary structures of these proteins are provided by circular dichroism and fluorescence measurements, respectively. Circular dichroism reveals little evidence for regular secondary structure in both molecules, since the optical activity is unusually low^{72,138}. Nevertheless, some folded regions appear to be present, since the tryptophan fluorescence spectrum suggests that at least some of the tryptophan residues are buried in hydrophobic regions of structure¹³⁸. Heating exposes the tryptophan sidechains, and destroys fibronectin's agglutinating activity^{50,138}. The fibronectins therefore appear to be floppy, asymmetric molecules, with localised regions of folding that may be important for adhesive activity.

Direct comparisons of the structures of fibronectins isolated from plasma and culture medium (which contains cell surface fibronectin secreted by cells) reveal very similar isoelectric points, migration in SDS–polyacrylamide gels, and cyanogen bromide cleavage products for proteins from these sources¹⁴⁵. However, there are several reports of slight differences in electrophoretic mobility between cellular and plasma fibronectins^{37,67,139}, and in some gel systems, the plasma form can be resolved as a doublet, whereas the cellular form is a single broad band^{20,24,26,37,172}.

The solubilities of the two proteins also differ. The plasma form is soluble at neutral pH²⁴, whereas the cellular form is insoluble unless the pH is elevated to pH 11 or it is treated with strong denaturing agents^{13,51,146,147}. The insolubility of cellular fibronectin may account for its aggregated distribution on the cell surface.

What accounts for these differences in solubility and electrophoretic mobility? Differences may exist in the amino termini of these proteins. For example, the differences in electrophoretic mobilities between these proteins are preserved even after the putative carboxyl terminal disulphide bonds are cleaved from the molecule by limited proteolysis with trypsin¹⁷². Human and bovine plasma fibronectins have amino terminal pyroglutamate residues^{24,26}, whereas chick cellular fibronectin does not⁵¹. One current speculation is that the two forms of fibronectin may be the same gene product, but the surface form is proteolytically processed to the slightly smaller plasma form. Alternatively, they may be produced by separate genes arising from gene duplication. Several laboratories are actively investigating these possibilities.

Carbohydrates of the fibronectins

The carbohydrates on cell surface fibronectin consist primarily of the 'complex' type of oligosaccharide linked by *N*-glycosidic bonds to asparagine. There are probably an average of 4–6 such oligosaccharide groups per molecule, containing terminal galactose, sialic acid, and fucose residues^{51,148,149}. The structure of the carbohydrate moieties of the plasma form of fibronectin are not known, although the compositions of the two forms are similar^{24,51,145,149}.

Treatment of cells with the antibiotic tunicamycin specifically blocks the formation of this class of oligosaccharide. Chick fibroblasts treated with tunicamycin produce unglycosylated fibronectin, and total amounts of this protein are substantially decreased^{148,150}. The decrease is not due to altered synthesis or secretion, but instead to increased proteolytic degradation¹⁴⁸. These results suggest that for cellular fibronectin, carbohydrates are not necessary for synthesis, processing, or secretion, but instead may function to protect the protein from proteolytic attack by cellular proteases.

Regulation of fibronectin

Quantities of cell surface fibronectin vary as cells traverse the cell cycle. Fibronectin levels are lowest on cells in mitosis by both radioactive surface labelling and immunofluorescence criteria, even though intracellular levels of fibronectin do not appear to vary significantly^{19,43,86,93}. This finding is of interest, since cells in mitosis also resemble transformed cells with respect to morphology, agglutinability by lectins, glycopeptide size, and loss of contact inhibition of movement¹⁵¹.

Even though fibronectin is decreased on cells during mitosis, the turnover rate of cell surface fibronectin is substantially longer than the cell cycle in chick cells (half remains after 36 h with an 18 h generation time; refs 55, 152; see also refs 21, 77, 86, 153). This apparent paradox could be explained if cells temporarily release themselves from the matrix of cell surface fibronectin during mitosis, and spread back into and rebind the same matrix as they re-flatten following cell division.

Growth conditions also influence quantities of cell surface fibronectin. For example, nonproliferating cells in dense cultures in the G1 phase of the cell cycle generally have larger amounts of fibronectin than rapidly growing cells^{15,19,47,83,85,86,93,98}. Cells prevented from dividing by serum deprivation can have either elevated¹⁹ or substantially reduced amounts of cell surface fibronectin¹⁵⁴. Mouse 3T3 cells cultured in media containing lowered concentrations of serum have little fibronectin; cell surface fibronectin levels are restored after treatment with epidermal growth factor, but not by other hormones¹⁵⁴. Fibronectin production by human fibroblasts is also reportedly stimulated by thrombin¹⁵⁵.

How fibronectin becomes depleted on the cell surface after malignant transformation has been studied in detail. Increased cell proteases after transformation cannot alone account for the loss of fibronectin^{84,87,156}. In chick fibroblasts transformed by several strains of tumour virus, the major factor in the decrease was found to be decreased biosynthesis⁵⁵, apparently due to decreased amounts of translatable messenger RNA¹⁵⁷. However, other factors also contribute to the decreases in fibronectin, including an increased rate of proteolytic degradation^{21,55,77} and a decreased capacity to bind the protein to the cell surface⁵⁵. In hamster cells, major factors in the loss of fibronectin after transformation also appear to be reduced biosynthesis and increased degradation^{107,158}. However, in human transformants, decreased capacity to retain the protein on the cell surface may be more important than its decreased synthesis^{30,52}.

Amounts of fibronectin on the cell surface are also regulated during embryonic differentiation. As *in vivo*⁵⁹, differentiation of muscle or cartilage cells *in vitro* is accompanied by a decrease in fibronectin on the cell surface^{47,159,160} (contrast ref. 98). Dedifferentiation of chondrocytes results in increased amounts of fibronectin^{160,174}. Experimental addition of cellular fibronectin to chondrocytes results in cells morphologically similar to undifferentiated mesenchymal cells that no longer synthesise the large amounts of sulfated proteoglycans characteristic of differentiated chondrocytes¹⁷⁵. Programmed losses of cellular fibronectin may, therefore, be a prerequisite for differentiation of certain cells.

These studies on the regulation of fibronectin levels on the cell surface indicate that control can be exerted by a variety of events including altered growth conditions, transformation, hormonal stimulation, and embryonic differentiation. This regulation may occur through altered synthesis, turnover, or binding to the cell surface.

Relationship of fibronectin to cytoskeletal and membrane components

The overall organisation of cell surface fibronectin into fibrillar patterns parallel to the long axis of cells is roughly similar to that of intracellular microfilament bundles⁴⁰. However, immunofluorescence studies comparing fibronectin and actin localisation patterns have shown that the two patterns

need not coincide (ref 32 and our unpublished results). These findings suggest that there may not be direct transmembrane connections between microfilament bundles and all of the surface fibronectin.

However, indirect associations or discrete attachment points may occur. For example, cell surface fibronectin is concentrated on the lower surface of cells¹⁶¹, and particularly in the adhesive attachment sites of cells to substrata¹⁶². Treatment of cells with low concentrations of proteases or anti-fibronectin can result in cell rounding and in a loss of microfilament bundle organisation^{34,163}. Treatment of transformed cells missing microfilament bundles with fibronectin permits reorganisation of intracellular microfilament bundles^{56,104}, perhaps due to increased cell-substratum adhesion that permits anchoring of these bundles (Fig. 3).

Conversely, cytoskeletal elements may also affect the organisation of cellular fibronectin. Treatment of cultured cells with cytochalasin results in a release of fibronectin from the cells, suggesting a possible requirement for microfilament integrity in maintaining fibronectin organisation^{164,165}. Treatment of cells with anti-fibronectin can result in antibody-induced redistribution of fibronectin¹⁶⁶ to 'caps' of antigen on the upper surfaces of cells³⁴. This process requires metabolic energy, and is inhibited by a combination of colchicine and cytochalasin³⁴; cytoskeletal elements may also be involved in this redistribution. More detailed analyses of the ultrastructural and biochemical relationships of these proteins should provide further insight into fibronectin-cytoskeletal interactions.

Fibrillar networks of cell surface fibronectin are relatively immobile by comparison with mobility of other cell surface components. For example, receptors for concanavalin A move with a diffusion constant of $4 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$, while fibronectin's is $< 5 \times 10^{-12}$ (ref. 42). As cellular fibronectin is relatively immobile, it might inhibit the mobility of other components of the cell surface. The movement of gold particles on the upper surfaces of 3T3 cells is inhibited by fibrils of cellular fibronectin¹⁶⁷. In contrast, the mobilities of a ganglioside analog, a lipid probe, and a mixture of surface antigens are not affected by the presence of fibronectin fibrils⁴². It is therefore possible that fibrillar networks of fibronectin affect interactions of cells with large objects such as particles, cell processes, or substrata, whereas smaller membrane components remain unaffected. These results are consistent with the location of fibronectin fibrils close to, but not necessarily tightly apposed to, the plasma membrane.

Future prospects

Although we know the overall structure, location, and probable general functions of the fibronectins, many important questions remain. For example, although the fibronectins are adhesive proteins, it is not clear how they function *in vivo*—for example in organising the extracellular matrix, cell migration, wound healing, macrophage ingestion of colloids, hemostasis, and embryogenesis. Is this protein directly involved in inhibiting tumorigenicity or metastasis *in vivo*? Is its role in the reticulo-endothelial system confined to the removal of collagenous debris, or does it play a wider role in host defense^{168,169}? Other interesting problems concern the precise differences in structure and regulation of the plasma and cell surface forms of fibronectin, their possible relationships to other more specific adhesive molecules that appear at precise times in vertebrate embryonic development¹⁷⁰, and the biophysical mechanisms by which these proteins can bind to cells and to substrata.

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articles

The significance of magnetospheric research for progress in astrophysics

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Recent in situ measurements have led to a substantial revision of our picture of the magnetosphere and parts of the heliosphere. This concerns the character and distribution of electric fields and currents, the ways in which charged particles are energised, and the chemical composition of the magnetospheric plasma. This revision reflects the fact that real cosmic plasmas behave in fundamentally different ways than predicted by the traditional, idealised models used in magnetospheric physics and in astrophysics. The new understanding of the general properties of cosmic plasma gives us an improved basis on which to interpret astrophysical observations.

ASTRONOMY, and especially astrophysics, is in an interesting phase, where remarkable observational discoveries are being made. However, since astrophysical phenomena occur in remote regions, where *in situ* observations are not possible, and involve matter in various extreme states, it can be difficult to understand the physical phenomena behind them. Usually the states involved are magnetised plasmas at extremes of temperatures and/or density. The interpretation of the observations then depends on whatever knowledge of plasma processes is available.

The difficulties involved can be understood by recalling those faced by thermonuclear fusion research 20 years ago. The task was to predict the behaviour of a very hot, magnetically confined, plasma. A well developed theoretical formalism existed. It was the classical theory of gases, generalised to include charged particles, but it had not been subjected to a thorough experimental verification. It was found that the hot plasmas in thermonuclear machines often behaved quite differently from what had been predicted from existing plasma theories. This necessitated a fresh start, with experiments as the backbone of the theoretical structures. However, even now the complicated behaviour of hot, magnetically confined plasma is far from being completely understood.

In astrophysics the situation is more difficult because, in contrast to the plasmas in thermonuclear machines, *in situ* 'diagnosis' of the astrophysical objects is not possible. The coming of the space age means that cosmic plasma can now be studied *in situ*, but only in our near-space environment.

To understand the significance of this, it is important to realise that our immediate environment in space, the magnetosphere (including the ionosphere) of the Earth and the adjacent interplanetary space, offer a rich sample of plasma

states in natural conditions. They include cool, weakly ionised, collision-dominated, gravity confined plasmas as well as hot, essentially fully-ionised and virtually collisionless magnetically confined plasmas. While the parameters may still be many magnitudes less than the extreme values attained in some remote astrophysical plasmas, the plasmas in the magnetosphere are a large step from the parameter range available in Earth-based laboratories. Study of the magnetospheric plasmas can, therefore, greatly widen the empirical basis of cosmic physics.

Spacecraft observations in the magnetosphere have already identified various plasma processes which were not predicted from classical kinetic theory, the MHD formalism or even modern plasma theories. In particular, as we point out below, limitations have been found to the validity of the classical concept of the frozen-in-magnetic field, which used to be a 'golden rule' in cosmic electrodynamics.

The need for an improved empirical basis in cosmic physics is dramatically illustrated by the history of magnetospheric research itself. Before *in situ* measurements were possible, and even for many years after satellite measurements had begun, the magnetosphere was described in terms of highly idealised models that were a heritage from classical plasma physics. They involved infinite conductivity along magnetic field lines, frozen-in-magnetic fields and simple magneto-hydrodynamic convection. It was tacitly assumed that the matter in the outer magnetosphere was (mainly) hydrogen ions from the solar wind, and that electric fields—if they were considered at all—were everywhere transverse to the magnetic field.

For a long time even spacecraft observations failed to reveal the fallacy of such assumptions, this was probably because their indiscriminant use guided the planning and design of the observations. Thus, for example, particle detectors in low Earth orbit always looked upwards because accelerated particles coming from below—as have been recently observed—were inconceivable in the prevailing theories.

Only during the past few years, has the magnetospheric physicist found that the classical concepts break down in certain interesting regions of the magnetosphere. Also, only during the past few years, have the significance of Birkeland currents (magnetic-field-aligned electric currents in the magnetosphere) and magnetic-field-aligned electric fields been realised. This has far-reaching consequences in understanding the aurora and other phenomena in the magnetosphere.

Although the understanding of the magnetosphere has increased gradually a veritable breakthrough has resulted from a few particle and field measurements on one single satellite,

S3–3. We now know that: (1) the frozen-field condition is not reliable, and different levels of the magnetosphere can be electrically decoupled—at least partially; (2) magnetic-field-aligned electric fields with potentials of several kV exist not only in narrow regions but over hundreds of km in latitude; (3) ionospheric ions are accelerated along the geomagnetic field into the magnetosphere; and (4) large, sometimes dominant part of the storm time ring current consists of oxygen from the ionosphere rather than hydrogen from the solar wind.

Such discoveries in magnetospheric physics indicate that the really important phase of magnetospheric research, in terms of fundamental physical understanding of plasma behaviour in natural conditions, has only just begun.

This will also have far-reaching consequences for the foundations of cosmic plasma physics and hence for astrophysics. If a cosmic plasma system which is so close to us could, for such a long time, be misinterpreted in terms of the physical processes going on, how confident can we be of the interpretations of the astrophysical phenomena known only by remote observations? Kelvin estimated the age of the Earth, on the basis of its cooling rate, to be 50 Myr because radioactivity was not known during his generation. Inadequate plasma physical models could lead to—and have already led to—similarly misleading conclusions in astrophysics.

It is worth emphasising the importance of laboratory experiments in cosmic plasma physics. In thermonuclear research a great deal of progress has been possible, after the initial setback, because a large experimental effort has guided the theoretical development. Similarly, in cosmic plasma physics, laboratory experiments, as well as magnetospheric research, are needed to guide the theoretical interpretation of remote observations. One example is the electric double layer phenomenon, which according to recent observations is likely to occur in the magnetosphere and may have profound consequences in cosmic plasmas. Although its existence has long been known in unmagnetised plasma, and recently proved in magnetised plasma, an adequate theory—especially of its dynamics—does not yet exist, and in all applications one must rely on results of laboratory experiments.

The role of magnetisation

Virtually any known astrophysical plasma is magnetised. To be precise, a magnetised plasma is defined as a plasma that contains a magnetic field which is strong enough to make the gyroradii of all its charged particles much smaller than the linear dimensions of interest. As the latter are usually large in cosmic plasmas, even a small magnetic field is sufficient to make the plasma magnetised.

For example, for stellar dimensions and temperatures of the order of 10^6 K, as in the corona, magnetic fields above 10^{-12} T are sufficient. For galactic dimensions, and even if we consider the highest observed energies, the corresponding number is 10^{-17} T. The smallest cosmic magnetic fields which can be measured or inferred are of the order of 10^{-9} T.

For magnetised plasmas it is often useful to distinguish between three classes: high, medium and low density plasmas¹. They are defined in terms of the relative magnitudes of the gyroradius, ρ , the mean free path, λ , and the characteristic linear scale, l_c , as follows:

High density, $\lambda \ll \rho$; medium density, $\rho \ll \lambda \ll l_c$; low density, $l_c \ll \lambda$.

High density plasmas occur in stellar bodies, lower stellar atmospheres and lower planetary ionospheres. Except for wave propagation they have a high degree of isotropy. Current densities and electric fields obey a simple local relationship—Ohm's law.

Medium density plasmas prevail in fairly thin transition regions above the lower stellar atmospheres and planetary ionospheres, but can also occur in regions such as the Earth's plasmasphere. These plasmas are anisotropic in terms of

diffusion and electric current conduction. The electric field–current density relation is still local and can be expressed as a generalised Ohm's law.

Low density plasmas prevail throughout most of the Earth's (and other) magnetosphere(s), the solar corona, interplanetary, interstellar and intergalactic space. They are the most complicated plasmas, because collective interactions tend to be important instead of the relatively simple binary collisions that prevail in low and medium density plasmas. Numerous kinds of plasma instabilities and wave–particle interactions are possible. In terms of electric properties, a major complication is that there need no longer exist any local relationship between electric field and current density. The reason is that charge carriers may gain momentum from the electric field in one region and lose it, for example, by magnetic mirroring, in a different region. Low density plasmas in this sense are a major preoccupation of modern plasma physics, especially in thermonuclear research where $\lambda \gg l_c$ is one of the requirements for magnetic confinement.

In the magnetosphere, the most exciting discoveries in recent years are related to the properties of low density plasmas. The consequences for the understanding of cosmic physics in general can be expected to be far reaching.

Electric currents and fields in cosmic plasmas

The magnetisation of all cosmic plasmas is connected with electric currents through Maxwell's first equation $\text{curl } \mathbf{B} = \mu_0 \mathbf{j}$ and as cosmic magnetic fields are not generally curl-free, a fundamental problem is how electric currents are carried by cosmic plasmas².

For high and medium density plasmas this is a simple problem, because the current is determined essentially by a local balance between electric, magnetic and frictional forces. The current-carrying ability of the medium at each point is characterised by a conductivity which in high density plasmas is a scalar, in medium density plasmas a tensor.

In low density plasmas the conduction of current is complicated. This fact is often overlooked, and classical conductivity formulas are erroneously applied to low density cosmic plasmas. One illusion is that, as collisions become rare and vanish, the conductivity along magnetic field lines increases and becomes essentially infinite. In reality, the concept of conductivity will lose its meaning¹. There need not be any local relation such as Ohm's law.

Magnetospheric spacecraft observations in the past few years have shown that the conductivity concept is obviously misleading. This has great consequences in many astrophysical applications, too. In particular, the ability of a low density plasma to carry electric current is far from being 'infinite', and substantial electric fields can be required to drive the Birkeland currents (magnetic-field-aligned currents) of the magnitude (of the order of 10^{-6} A m⁻²) observed in the magnetosphere. This greatly affects astrophysics, because first it changes the whole electrodynamics of cosmic plasma; second, the finite electric field along magnetic field lines violates the concept of frozen-in magnetic field lines, and finally it provides an efficient means of particle acceleration.

A few typical phenomena will now be briefly described that involve substantial magnetic-field-aligned electric fields.

Anomalous resistivity has been recognised for over a decade as a possible means of supporting magnetic-field-aligned electric fields in space plasma (for a review see ref. 3). Anomalous resistivity is characterised by wave-turbulence sufficiently strong to impede the current-carrying electrons so that a local Ohm's law is reestablished. The wave turbulence is usually considered to be driven by the current itself, but may also be maintained by a separate agent such as a high-energy electron beam that is unstable in the local plasma environment^{4–6}. Whatever the cause may be, the d.c. electric field strength, E , that can be supported is limited by the condition $E \ll E_{rms}$,

where E_{rms} is the r.m.s. value of the electric field of the wave turbulence⁷. This has been confirmed by direct measurements in the magnetosphere⁸.

The wave turbulence will act differently on particles in different parts of velocity space. Particles of sufficient initial velocity (in the direction of the electric force) will become runaways, that is, decoupled from the main population of charge carriers, and the current they carry will not be determined by local conditions but depend on the whole electric circuit involved. Therefore, if the runaway current becomes substantial compared to the total electric current, the local relationship between electric field and current is lost. Thus the current is not determined by local 'resistivity', and the whole circuit must be taken into account. Then the term 'resistivity' loses its meaning.

The anomalous resistivity phenomenon has been established by laboratory experiments (for a review, see ref. 9) and wave fields measured in the magnetosphere (for a review, see refs 10, 11) are large enough to support magnetic-field-aligned electric fields of the order of $mV m^{-1}$, which may integrate to voltage drops of the order of several keV, a field large enough to be of significance to magnetospheric plasma dynamics. On the other hand, in the magnetosphere, anomalous resistivity may not be the only, or even the most important, mechanism for supporting magnetic-field-aligned electric fields. Alternative mechanisms are the double layer and the magnetic mirror effect.

The electric double layer was discovered by Langmuir in 1929 but wide interest in the phenomenon has been revived recently by experiment studies¹²⁻¹⁴. For cosmical applications it is essential that its existence in magnetised plasma has now been proved^{15,16}. (For a review with special reference to possible space applications, see refs 17, 18.)

Like anomalous resistivity, a double layer is typically established as a consequence of current-driven plasma instabilities. To predict whether the end result of a given instability will be anomalous resistivity or a double layer is difficult. Theoretical studies have therefore often started with the assumption that the end state is one of anomalous resistivity.

The laboratory experiments which show that the result of the instability may well be a double layer instead of anomalous resistivity, illustrate the danger of a purely theoretical approach and the need for empirical experiments on the behaviour of the real plasma.

The electric potential drop across a double layer depends on factors such as density and temperature of the adjoining plasmas and on the electric properties of the circuit in which the current is closed. Thus, unlike anomalous resistivity, the electric double layer does not imply a local relationship between electric field and current density.

Double layers and anomalous resistivity differ in the way that the dissipated energy is deposited. In the case of anomalous resistivity, it is deposited in the ambient plasma at the location of the voltage drop, leading to intense local heating there. In the case of a double layer, all particles passing through the layer are 'runaways', and form beams that carry away the dissipated energy to be deposited in some other region where the beam is finally stopped. In the magnetosphere, this may account for the energy deposition in the polar ionosphere by auroral primaries without excessive heating in the acceleration region. In many astrophysical applications such remote deposition of energy may be very important.

Direct observations of double layers are difficult because they are relatively thin, 10–100 Debyelengths. The probability of encountering a double layer is one in several hundred satellite passes. In the recent electric field data of the S3-3 satellite⁸ there is one case (from about 200) where the magnetic-field-aligned electric field was reported to be several hundred $mV m^{-1}$. This is compatible with an electric double layer¹⁹.

In a low density plasma situated in a magnetic mirror field slight differential anisotropies between positive and negative

particles can lead to large magnetic-field-aligned electric fields even without a net current¹. In the magnetosphere where large field-aligned currents (Birkeland currents) flow to and from the auroral oval, and in many astrophysical situations, the magnetic mirror effect puts an upper limit to the conductance per unit area—a conductivity need still not exist—which above the auroral region of the Earth is at most of the order of a few $A m^{-2} keV^{-1}$ (refs 7, 20). This requires that the loss cone of the source plasma is continually replenished. If it is not, the current will be choked and the conductance reduced to still smaller values and can in principle approach zero.

The frozen field condition

If the classical conductivity formula were applicable to cosmic plasmas, the condition for 'frozen-in magnetic fields' would almost always be fulfilled. But the plasma properties described above may well decouple the electric fields in different portions of a magnetic flux tube (magnetic field unfreezing). This occurs at least in a limited region of the magnetosphere above the auroral ovals. Recent observations of comet tails²¹ are apparently the first proof of magnetic-field unfreezing in plasmas outside the Earth's magnetosphere.

Magnetic field unfreezing may also invalidate the law of isototation, and in the context of planet and satellite formation allow an important phenomenon called partial corotation²².

Particle acceleration

Cosmic plasmas can generate suprathermal and high energy particles. In ordinary particle accelerators, insulators and sometimes also tuned harmonic signals are requisites for accelerating charged particles to high energy. None of these requisites are available in cosmic plasmas. Yet we observe cosmic ray particles with energies of 10^{19} eV or more, solar protons of 10^{10} eV and magnetospheric protons of $1-10^6$ eV. It would be difficult to generate such high-energy particles if the plasma had the simple magnetohydrodynamic properties usually ascribed to it.

Some particle energisation processes in cosmic plasma can be studied in the magnetosphere. Their products range from slightly suprathermal electron fluxes in the ionosphere to ions of several MeV in the radiation belts and in the tail region of the magnetosphere. Some of these acceleration processes involve wave fields due to various instabilities. Others depend on slowly fluctuating transverse electric fields to cause drift-resonant adiabatic acceleration, which can be compounded with non-adiabatic effects. An interesting process is the direct acceleration of charged particles in the magnetic-field-aligned electric fields that regularly occur in low density plasmas. This possibility was previously excluded by the assumption that magnetic-field-aligned electric fields cannot exist and that the frozen-field condition is always valid. In the magnetosphere, such a direct acceleration process seems to play an important part in auroral phenomena^{7,17,18,23,24}. In the solar atmosphere the field-aligned acceleration may also play an important part in flare phenomena²⁵, which is widely recognised to have many similarities with magnetospheric substorms²⁶. Field-aligned acceleration may also be important in many other cosmic plasmas. One example on a large linear scale is double radio sources²⁷. The energy, originating in the unipolar dynamo of the central galaxy, is carried by electric current to the two opposite remote regions where the current becomes unstable and a parallel electric field is established (presumably in a double layer).

The structure of matter in space

One of the most important results of space exploration is the discovery of a number of boundary surfaces. Some of these layers are due to electric sheet currents which may reverse the magnetic field. This often occurs in the magnetopause, in the neutral sheets of the magnetotail, in the solar equatorial plane² (previously interpreted as 'the sector structure'), and in

cometary tails²¹. Such boundary sheets may be very thin, sometimes only a few Larmour radii. Their persistence demonstrates that they have a high degree of stability and that cosmic plasmas have a general tendency to divide themselves into compartments by setting up such layers.

The discovery of the many boundaries separating different plasmas is remarkable in that neither theoretical considerations nor laboratory experience had led us to expect layers with the observed properties (although other types of layers were predicted). This is very important for our picture of the structure of space. We find that space occupied by plasmas tends to be divided in 'compartments', often separated by thin current sheets. On the interstellar and intergalactic scale, space may have a cellular structure consisting of many separate regions, containing plasmas of different magnetisation, density, temperature, and perhaps also different kinds of matter (koino- and antimatter).

Conclusions

For a proper understanding of astrophysical phenomena, the theoretical work must be guided by empirical knowledge of how plasmas behave in nature. Laboratory experiments have an essential role in this context but *in situ* observations of the magnetospheric plasma can, in some respects, provide us with an even better knowledge of plasma behaviour in natural conditions. The plasma in the magnetosphere (including the ionosphere) and the solar wind are the only cosmic plasmas accessible to extensive *in situ* observations and experiments.

Observations of magnetospheric plasmas extend our empirical knowledge to a new range of plasma parameters by many powers of 10. It is also fortunate that the Earth's magnetosphere contains such a rich variety of plasmas, from the relatively dense and cool ionospheric plasma to the extremely thin and hot plasmas at larger distances.

The truly fundamental progress in the understanding of the

magnetosphere has only just begun. It has begun because of the recent observational discoveries which mark a new phase of magnetospheric research. The knowledge already obtained, and the insights still to be gained, in this new phase of magnetospheric research should be of great value in understanding astrophysical plasmas and may lead to similar drastic revisions in astrophysics.

Received 8 May 1978; accepted 29 Jun 1978.

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Young's double beam interference experiment with spinor and vector waves

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The fringe shift observed in Young's double beam interference experiment using unpolarised neutrons, when one beam traverses a magnetic field before recombination, has been interpreted as implying direct observation of the sign reversal of a spinor wave function subjected to a 2π rotation. This interpretation is unsatisfactory because for any fermion the relative rotation of the spins in the two beams and the interference pattern are mutually incompatible observables. This incompatibility does not arise in the case of bosons which possess a state of zero spin-component. Furthermore, equivalent 'sign-reversal' effects may be observed in interference phenomena associated with any two-state system and a realisable photon analogue is proposed.

RAUCH ET AL.¹ and Werner *et al.*² have independently described neutron analogues of Young's double slit optical interference experiment using unpolarised neutrons which have been interpreted as a direct observation of the sign rever-

sal of a fermion wave-function under a 2π rotation. In these experiments, amplitude division of a coherent neutron wave train is brought about by a symmetrical Laue reflection in a single crystal of silicon. One of the emergent beams traverses a uniform magnetic field while the other propagates in a field-free region. The crucial result is the observation that the interference pattern formed where the two beams recombine, shifts through half a fringe when the magnetic field is varied from zero to a value such that the spins of those neutrons moving through the magnetic field precess through an angle of 2π relative to the spins of those neutrons which move in the field free region^{3,4}.

Interference pattern

This extremely elegant technique provides an interferometric method of distinguishing fermion from boson⁵ because the equivalent boson interference pattern would shift through a full fringe. One must, nevertheless, be cautious about the claim² that "we have directly observed the sign reversal of the wave function of a fermion produced by its precession of 2π radians in a magnetic field using a neutron interferometer". The difficulty relates to the notion of 'relative rotation'³⁻⁷ as applied

to the fermion spins in the context of an interference experiment as, for fermions, simultaneous observations of the relative rotation of the spins in the two beams and of the phase shift are incompatible. We may, of course, measure the angle of rotation of those neutron spins constrained to move in the magnetic field relative to the spins of neutrons in the field-free region. An essential feature, however, of all interference phenomena is that, whereas the wave amplitudes propagate along distinguishable paths, all information concerning which path the neutron actually follows is lost⁸. In these circumstances the notion of relative rotation ceases to have a meaning as it corresponds to nothing which is measurable. The objection therefore is founded on the uncertainty relation and is not pedantry because, as we show below, no equivalent inconsistency arises in the case of bosons.

Let $\mathbf{u}_0$ be the incident spinor, then the neutron wave train reaching the detector in the absence of the interferometer is represented by the spinor $\mathbf{u}_0(x) = \mathbf{u}_0 e^{ip_0 x/\hbar}$. When the interferometer is introduced $\mathbf{u}_0(x)$ is transformed to

$$\mathbf{u}_i(x) = \begin{bmatrix} A + B e^{-i\beta/2} & 0 \\ 0 & A + B e^{i\beta/2} \end{bmatrix} \mathbf{u}_0(x) = M \mathbf{u}_0(x) \quad (1)$$

where A and B are real parameters characterising the amplitudes of the interfering beams and $\beta = -geBx/2p_0$ (ref. 3). The action of the interferometer is described by the transfer matrix

$$M = \begin{bmatrix} T^{1/2} e^{-i\alpha/2} & 0 \\ 0 & T^{1/2} e^{i\alpha/2} \end{bmatrix} \quad (2)$$

where T , the matrix determinant, is given by

$$T = A^2 + B^2 + 2AB \cos(\beta/2) \quad (3)$$

and

$$\tan\left(\frac{\alpha}{2}\right) = \frac{B \tan(\beta/2)}{B + A \sec(\beta/2)} \quad (4)$$

As M describes a transformation to a state whose normalisation has been multiplied by $T^{1/2}$, we may interpret T as the transmission coefficient for the interferometer. It follows from equation (3) that the interference pattern has a minimum at $\beta = 2\pi$ and the fringe visibility V is therefore

$$V = 2AB/(A^2 + B^2) \quad (5)$$

The observation of the interference pattern exhausts the experimental possibilities for unpolarised neutrons.

Because there is no uncertainty relationship between spin and position it is possible, using polarised neutrons, to make simultaneous measurements of the interference pattern and of the neutron spin polarisation at each point along it. The additional information may be derived from the density operator ρ which according to equation (2) is transformed to

$$\rho_i(x) = M \rho_0(x) M^{-1} = \begin{bmatrix} \rho_{11}^0 & \rho_{12}^0 e^{-i\alpha} \\ \rho_{21}^0 e^{+i\alpha} & \rho_{22}^0 \end{bmatrix} \quad (6)$$

The polarisation $\mathbf{P}_i$ of the transmitted beam is defined by

$$\mathbf{P}_i = \langle \boldsymbol{\sigma} \rangle_i = T_i(\rho_i(x) \boldsymbol{\sigma}) / T_i(\rho_i(x)) \quad (7)$$

and in particular

$$P_{zi} = P_{z0} \quad (8a), \quad (P_x + iP_y)_i = (P_x + iP_y)_0 e^{+i\alpha} \quad (8b)$$

The essential results are contained in equations (8) showing that although the component of the neutron polarisation in the recombined beam parallel to the magnetic field is unchanged from what it was before the division of amplitude, the polarisation vector in the plane transverse to the magnetic field is rotated through an angle α . Thus, although the density matrix contains sufficient information to allow us to make predictions about the absolute rotation of the neutron spins in the recombined beam it contains no information about the relative rotation of the spins in the constituent beams. The essential point is, of course, that whereas the phase difference β between the

spatially separated wave trains is determined by the magnetic field and the passage time alone, the absolute angle of rotation α of the polarisation vector in the reconstituted beam also requires for its specification the mixing ratio B/A of the component wave trains as expressed through equation (4).

To emphasise the significance of these results from an experimental viewpoint we might envisage carrying out two limiting types of measurement. In the first case we set $A < B$ and determine the spin rotation of the transmitted neutrons which reaches the value π at $\cos \beta/2 = -A/B$, making the transition to 2π at $\beta = 2\pi$. Alternatively we can set $A > B$ in which case α achieves a maximum deviation of $2 \tan^{-1}[(B/A)/(1 - (B/A)^2)^{1/2}]$ at $\cos \beta/2 = -B/A$ and then drops rapidly back to zero at $\beta = 2\pi$. Thus the polarisation in the recombined beam carries out a rotation for $A < B$ and a nutation for $A > B$. There is no 'relative rotation'. When either A or B tends to zero the polarisation approximates to that of neutrons whose paths are constrained but the fringe visibility reduces in proportion.

At the other limit we set $A = B$ with unit fringe visibility and, as $\beta \rightarrow 2\pi$, we find that $\alpha \rightarrow \pi$ and the polarisation in the transmitted beam is reversed. Evidently there is a discontinuity in α at $\beta = 2\pi$ in the transition from $A < B$ to $A > B$ which means that at maximum fringe visibility the spin rotation becomes indeterminate at the interference minimum.

Apparently we must omit the case $A = B$, representing a fringe visibility of unity and total polarisation reversal, because in this situation the transfer matrix is null at the interference minimum and no neutrons are transmitted. However, this results from a defect in the formalism which uses plane wave states; in the more realistic wave packet description the factor $\cos \beta$ in equation (3) is replaced by the real part of, and the visibility $V < 1$ by the modulus of, the complex degree of coherence in the interfering wave trains, thus the reduction of the transfer matrix to singular form is avoided.

Bosons

The equivalent situation for bosons may be explored by treating the case of a spin 1 particle whose spin wave-function is characterised by three independent amplitudes a_+ , a_0 , a_- , corresponding to states with spin component $M = 1, 0$ or -1 along the magnetic field. The density matrix now has 9 elements which may be organised into subsets of 1, 3 and 5 elements which are the expectation values of spherical tensors of rank 0, 1 and 2 respectively^{9,10}. The first rank tensor moments are just the components of the spin polarisation.

$$P_z = \langle |a_+|^2 - |a_-|^2 \rangle / I \quad (9a)$$

$$P_x \pm iP_y = \langle a_+^* a_0 + a_+ a_0^* \rangle / \sqrt{2} I \quad (9b)$$

where $I = |a_+|^2 + |a_0|^2 + |a_-|^2$ is the total intensity.

In the presence of a magnetic field the amplitudes $a_{\pm}$ are shifted in phase to $a_{\pm} e^{\mp i\beta}$ and it should be noted from equation (9) that the transverse polarisation is linear in these amplitudes and their complex conjugates. The amplitude a_0 is unaffected by the magnetic field and this is crucial to the analysis since it follows that the transverse polarisation in the recombined beam does not contain any interference terms derived from products of field-dependent amplitudes. For spin 1 particles, equations (8) therefore become

$$P_{zi} = P_{z0} \cdot (A^2 + B^2 + 2AB \cos \beta) / T \quad (10a)$$

$$(P_x \pm iP_y)_i = (P_x \pm iP_y)_0 ((A+B) \cdot (A+B e^{\pm i\beta})) / T \quad (10b)$$

where in this case the transmission

$$T = A^2 + B^2 + 2AB \cos \beta + 2AB(1 - \cos \beta)(|a_0|^2/I)_0 \quad (11)$$

is sensitive to the degree of alignment $|a_0|^2/I$ in the beam incident on the interferometer.

Equation (10b) exhibits the fundamental feature that the term $(A+B) \cdot (A+B e^{\pm i\beta})$ may be represented as the incoherent sum of a fixed polarisation $(A+B)A$ and a rotating

polarisation $(A+B)B e^{+i\beta}$ whereas for spin $\frac{1}{2}$ fermions there is a single (rotating) term derived from the coherent superposition of field dependent amplitudes. The weights $(A+B)A$ and $(A+B)B$ are identical with the weights A^2 and B^2 obtained by incoherently superimposing the constituent beams only, for the case of maximum fringe visibility but this is an insignificant point as the presence of the two terms allows us to give an operational meaning to the concept of relative rotation in the recombined beam whatever their relative magnitude.

To show that the concept of 'relative spin rotation' retains a meaning for bosons in the setting of an interference experiment we only have to examine the corresponding predictions for the two limiting experiments discussed above in the case of fermions. When $A \rightarrow 0$ and the fringe visibility becomes vanishingly small we find from equation (10) that the transmitted polarisation vector rotates through the angle β as expected. When $B \rightarrow 0$ there is no rotation and the angle β between the two orientations of the analysing axis of a filter giving maximum transmission for these two cases defines the relative rotation in the absence of interference. At the opposite extreme, where $A \approx B$ and the fringe visibility is maximum, there are two groups of neutrons, with relative intensities $(A+B)A$ and $(A+B)B$ respectively, that is those whose spins have suffered no deviation and those whose spins have rotated through the same angle β . We can again measure the relative rotation, the same relative rotation β as for the case of zero interference, by observing the two directions of the analysing axis of the filter which permits free passage to neutrons. This is quite different to the fermion case where there is transmission at a single angle $\alpha \neq \beta$ only and no method of measuring a relative rotation.

Not surprisingly we find from equation (11) that the intensity in the interference pattern for bosons reaches a maximum when $\beta = 2\pi$. The minima in the interference pattern occur at $\beta = \pm\pi$ and it is interesting that the transverse polarisation vanishes at the minima, when conditions are such as to maximise the fringe visibility.

To examine the behaviour of the vector polarisation does not exhaust the possibilities where spin 1 particles are concerned because the second rank tensor moments are also experimentally accessible quantities. Denoting these moments by $t_{j,M}$ (refs 9, 10) we find

$$\begin{aligned} (a) \quad t_{2,0} &= (1/\sqrt{2})(|a_+|^2 - 2|a_0|^2 + |a_-|^2) \\ (b) \quad t_{2,\pm 1} &= (\sqrt{3}/2)(a_+ a_0^* - a_-^* a_0) \\ (c) \quad t_{2,\pm 2} &= \sqrt{3} a_+^* a_- \end{aligned} \quad (12)$$

Clearly the magnetic field-dependence of the moments $t_{2,0\pm 1}$ is the same in essential respects as that of the moments $t_{1,0\pm 1}$ whose cartesian components are given in equation (9). The tensor moments $t_{2,\pm 2}$ behave quite differently in a magnetic field in that they are shifted in phase by $e^{\pm 2i\beta}$ when the amplitudes $a_{\pm}$ collect phase factors $e^{\pm i\beta}$. Therefore $t_{2,\pm 2}$ revert to their original values when $\beta = \pi$ and the amplitudes $a_{\pm}$ change sign. Thus $t_{2,\pm 2}$ are totally analogous to the components of the vector polarisation of a spinor field.

Sign reversal effects

Clearly then where 'sign reversal' effects are sought, the number of states available to the particles is not crucial, the important thing is that two of the participating states should be phase shifted in opposite directions by the application of a suitable perturbation. This has been demonstrated by Klempt¹¹ who observed sign reversal effects for transitions between the hyperfine levels of the diatomic molecule TIF. The experiments were carried out on a molecular beam resonance apparatus using the Ramsey separated oscillatory coil technique. Thus, although both sign reversal and interference effects were generated in spin space the underlying principle was the same as for neutron double beam interference.

The distinction between the results derived for spin $\frac{1}{2}$ and spin 1 particles in the case of the vector polarisation has been

traced to the existence in the latter case of a state, the $M=0$ state, whose amplitude is insensitive to the presence of the magnetic field. It follows, therefore, that the spin $\frac{1}{2}$ results are characteristic of all fermions, whereas the spin 1 results are characteristic of all bosons of non-vanishing spin for which an $M=0$ state exists. The single known boson excluded by this criterion is, of course, the photon for which the spin state can be defined only with respect to the momentum with the $M=0$ state disallowed because of the vanishing rest-mass.

In the neutron interferometer the magnetic field causes the neutron spins in one arm to alter their state from positive helicity to negative helicity and back again. A photon analogue would require the role of the magnetic field to be filled by an optical element which converts right circular polarisation to left circular polarisation and back again to right circular polarisation. Two half wave plates in series perform precisely these functions and a full wave plate introduces a phase difference of 2π between the polarisation states exactly as a magnetic field does for the neutron spin-states. Indeed Bernstein, who first thought of the neutron interference experiment³ specifically compares the action of the magnetic field to that of a birefringent medium.

The active element in the interference experiment proposed above is a uniaxial crystal with its optic axis normal to the direction of propagation of the beam. If a_x and a_y represent the components of the complex vector potential parallel and perpendicular respectively to the optic axis then the amplitudes $a_{\pm}$ for states with spin component $M = \pm 1$ along the momentum are

$$a_{\pm} = \mp(1/\sqrt{2})(a_x \mp ia_y) \quad (13)$$

Thus the circular polarisation P_c in the beam is given by

$$P_c = i\langle a_x a_y^* - a_x^* a_y \rangle / I \quad (14)$$

where

$$I = \langle |a_x|^2 + |a_y|^2 \rangle \quad (15)$$

is the intensity.

The relationship between outgoing and incoming amplitudes in the light beam traversing the crystal may now be expressed as

$$\begin{pmatrix} a'_x \\ a'_y \end{pmatrix} = \begin{pmatrix} e^{i\delta_x} & 0 \\ 0 & e^{i\delta_y} \end{pmatrix} \begin{pmatrix} a_x \\ a_y \end{pmatrix} = e^{i\Delta/2} \begin{pmatrix} e^{i\delta/2} & 0 \\ 0 & e^{-i\delta/2} \end{pmatrix} \begin{pmatrix} a_x \\ a_y \end{pmatrix} \quad (16)$$

where

$$\begin{aligned} \delta_x &= \frac{2\pi}{\lambda} n_o z & \delta_y &= \frac{2\pi}{\lambda} n_E z \\ \Delta &= (\delta_x + \delta_y) & \delta &= (\delta_x - \delta_y) \end{aligned} \quad (17)$$

z is the path length in the crystal, and n_o and n_E are the ordinary and extraordinary indices of refraction.

It follows from equations (14) and (16) that, for a beam prepared in a state of pure circular polarisation P_c without ellipticity, the output circular polarisation P'_c is given by

$$P'_c = P_c \cos \delta \quad (18)$$

Thus, the circular polarisation is reversed when $\delta = \pi$, which described a half-wave plate. Two half-wave plates in series thus recover the original circular polarisation but change the signs of the amplitudes according to equation (16). The remainder of the theory proceeds as before and we can construct the analogue of the transfer matrix in equation (1) with β replaced by $-\delta$ and B by $B e^{i\Delta/2}$.

The observability of the photon amplitudes has no relevance here as we are concerned solely with observations on the Stokes operators¹² which characterise the polarisation properties of the beam and are quadratic in these amplitudes. The Stokes operators satisfy the same commutation rules as angular momenta and in view of the homomorphism of the group SU(2) on the group R3 any unitary transformation on a two

state system such as the photon has an analogous rotation for a spin $\frac{1}{2}$ fermion⁵. This justifies the photon analogue proposed above which is exact, provided the cycle of unitary transformations on the spin states of the photon is not interpreted as a rotation in real space. One can construct another photon analogue by using an optically active crystal to introduce a phase difference of 2π between the amplitudes $a_{\pm}$; in this case a real rotation of the plane of polarisation does take place but it is a rotation through an angle π rather than through an angle 2π (ref. 4).

To observe the minimum in the optical interference pattern obtained by introducing a full wave plate in one arm of an interferometer it would be necessary to eliminate the phase factor $e^{i\delta/2}$ in equation (16) by equalising the optical paths in both arms using a compensator according to normal optical practice. Two half wave plates in series with their optic axes at right angles would fulfil this role ideally. This requirement does not invalidate the principle of the device and indeed the same

defect exists in the neutron experiment (the 'residual phase shifts'³) although in a less severe form because the equivalent 'optical path difference' vanishes to first order provided the neutron energy is sufficiently low.

Received 19 April; accepted 10 July 1978.

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Mass mortality of photosynthetic bacteria as a mechanism for dark lamina formation in sediments of the Black Sea

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Black Sea seasonal entrainment of dissolved oxygen below the chemocline results in a temporary hiatus in which an oxygen induced mass mortality of photosynthetic bacteria initiates their mass sedimentation. During their sedimentation black coloured FeS-rich granules form. The abundance of these granules in the sediments dictates their colour and microlamination pattern.

RHYTHMICALLY laminated sediments from meromictic lakes and seas have long fascinated geologists, limnologists and oceanographers as these lamina have been used to construct detailed chronologies based on the supposition that a light and dark coloured band (couplet) represents events which repeated themselves on an annual basis.

Most of the sediments of the Black Sea are microlaminated¹ and these microlamina are thought to represent annual events or varves². On this basis a detailed chronology has been erected for the sediments of the Black Sea^{3,4}. Discrepancies between the ¹⁴C age and the varve count age for some of the recent Black Sea sediments¹ prompted us to examine these same sediments. An hypothesis concerning a mechanism for varve formation was formulated to explain the relative lack of correlation between the ¹⁴C and varve count chronologies in these microlaminated coccolith-rich sediments.

Recent reports⁵ have speculated that during both Cambrian and Precambrian times the majority of the oceans and seas of the world were meromictic, that is, permanently stratified into a deep non-mixing anaerobic layer termed the monimolimnion, and an overlying oxygenated mixing layer termed the mixolimnion. These conditions are similar to those currently found in the meromictic Black Sea⁵. To this extent the Black Sea

serves as a 'living fossil' and an understanding of the conditions responsible for dark and light coloured lamina formation there should assist us in interpreting the microlaminated deposits in the sediments of Cambrian and Precambrian seas.

The Black Sea is the largest 'lake' in the world⁶. The history of its tectonically formed euxinic basin, which now contains essentially marine waters, has included long periods when the basin was occupied by relatively fresh water⁷. We have therefore adopted the terminology of Hutchinson⁶ in describing this meromictic body of water. The water masses of the present day Black Sea are divided by a sulphide-rich chemocline into a lower saline anaerobic zone (monimolimnion) and an overlying less saline seasonally stratified mixolimnion.

During the last thousand years, a carbonate-rich microlaminated coccolith ooze has been deposited in anaerobic conditions at depths of 200 m or more along the bottom of the Black Sea^{1,2}. The detailed chronology of the Black Sea sediments discussed above was first formulated in the early thirties and forties^{3,4}. Many sediment studies of the Black Sea have been carried out⁷ since then but none have hinted at the possible impact of a mass mortality of photosynthetic bacteria on the microlamination pattern within its sediments. These bacteria are one of the main primary producers within the Black Sea⁸ and their annual mass mortality was not suspected until it was observed in another much smaller meromictic body of water in Quebec, Canada⁹.

The present study attempts to prove that oxygen-induced mass mortality of these photosynthetic bacteria (which are restricted to the anaerobic waters below 160 m)¹⁰ has been a major factor contributing to dark lamina formation in the Black Sea. Information is presented to show that the seasonal mass mortality of these photosynthetic bacteria occurs as a result of oxygen entrainment into the anaerobic waters which they occupy and that this deep-layer oxygenation is most likely

Table 1 Vertical profiles at station A for dissolved O₂, salinity, specific conductivity, water density and H₂S and bacterial abundance and biomass for stations A and B

No. of Replicates	Depth (m)	Oxygen* (p.p.m.)	Salinity* (PPT)	Conductivity* ($\mu\text{S cm}^{-1}$)	Temperature* (°C)	Density (sigma T)	H ₂ S	Bacterial† density at A	Bacterial‡ biomass at A	Bacterial density at B	Bacterial biomass at B
—	0.5	6.83	17.70	23.02	14.70	12.80	0	Photosynthetic bacteria were absent between 0 and 200 m		Photosynthetic bacteria were absent between 0 and 160 m	
—	10	7.26	17.90	23.04	14.72	12.95	0				
—	25	7.28	18.40	23.70	14.40	13.39	0				
—	50	8.40	18.10	21.40	9.80	13.88	0				
—	60	7.84	18.30	20.40	8.00	14.25	0				
—	75	6.50	18.90	20.36	7.40	14.78	0				
—	80	5.20	18.96	20.56	7.44	14.72	0				
—	85	3.60	19.20	20.90	7.44	15.01	0				
—	90	2.92	19.70	21.44	7.60	15.38	0				
—	100	2.18	20.00	21.90	7.90	15.59	0				
—	115	—	20.40	22.34	8.14	15.87	0				
—	125	0.61	20.54	22.46	8.65	15.92	0				
2	150	—	20.65	22.57	8.75	15.99	t§	0.7 (0.3/1.1)	0.35	1.2(0.8/1.6)	0.60
4	160	0.54	20.80	22.71	8.74	16.11	t§	0.6(0.2/0.9)	0.30	228(202/254)	114
3	165	0.54	20.86	22.77	8.79	16.15	t§	2.7(2.1/2.9)	1.35	1.8(1.2/2.1)	0.88
4	170	0.52	20.95	22.86	8.80	16.22	t§	8.2(6.7/11.2)	4.10	1.4(1.0/1.9)	0.71
2	175	0.38	21.00	22.91	8.87	16.25	t§	13.8(11.0/16.5)	6.9	1.2(0.9/1.5)	0.60
2	200	0.00	21.35	23.26	8.90	16.52	p§	160.0(11.8/20.3)	80	4.6(3.5/5.6)	2.30
2	225	0.00	21.70	23.60	8.97	16.78	p§	1.6(0.9/2.2)	0.8	1.3(0.7/1.8)	0.65

* Oxygen, salinity, specific conductivity, temperature and density were recorded only at station B. Bathythermograph results indicated a sharp thermocline beginning at 37 m (14 °C), 40 m (13 °C), 42 m (12 °C), 43 m (11 °C), 45 m (10 °C), 47 m (9 °C), 50 m (8.4 °C).

† Bacterial density at station A as numbers per ml times 10^3 with the maximum and minimum values ($\times 10^3$) recorded in parentheses.

‡ Bacterial biomass at station A as mg m^{-3} , calculated on the observed mean bacterial size of $0.5 \mu\text{m}^3$ per cell.

§ t, trace; p, present.

to occur during winter when partial destratification of the mixolimnion has occurred.

After the photosynthetic bacteria are killed by the addition of dissolved oxygen to their environment, they begin to settle slowly ($1\text{--}2 \text{ m h}^{-1}$)¹¹ towards the bottom. During their descent the elemental sulphur which they store as a waste product of their H₂S-based photosynthesis¹² is exposed to the ferrous-rich waters of the upper layers of the anaerobic zone¹⁰. The elemental sulphur once exposed is capable of acting as nuclei for the formation of black ferrous sulphide compounds. The presence of ferrous sulphides and their products (such as FeS₂ pyrite) produces dark-coloured microlaminae.

Experimental methods

Using a Simrad SK3 sonar unit deployed in the Turkish hydrobiology research vessel, R/S Arar, it was possible to detect and later sample the thermocline and chemocline at 29° 05' E, 41° 40' N at a depth of 830 m, 30 km from the Bosphorus sill near Kilyos, Turkey (station A). Nansen bottles and a 10-l stainless steel Petersen styled closing cylinder were

used to remove replicated water samples for immediate preservation and later bacteriological analysis. A simple Van Dorn sampler replaced the more sophisticated equipment mentioned above at the Trabzon, Turkey Station (39° 55' E, 41° 10' N) which was located in 800 m of water (station B).

Quantitative estimates of algal and bacterial densities were made using a Fein Optik haemocytometer and a Leitz Orthoplan microscope. The plankton from immediately above and below the H₂S chemocline (160 m at station A, and 200 m at station B) was preserved with Lugol's IKI solution and concentrated by simple sedimentation¹³. Salinity, specific conductivity and temperature were measured at station B using a Beckman R-55-3 electrodeless salinometer. Dissolved oxygen was estimated at the same station using a Winkler calibrated Wissenschaftlich-Technisch Werkstätten Oxidigi Meter (model 5500). Such equipment was not available in Trabzon.

Sediments from the Black Sea sampled during the Glomar Challenger deep sea drilling operation² were available for study. Black Sea sediments were analysed by scanning electron microscopy: samples were freeze fractured, affixed to SEM

Table 2 Abundance of black granules in light and dark coloured microlamina from the Black Sea core

Code* no.	Sediment colour	Sediment type	Sediment depth (cm)	Ferrous sulphide† black granules [(no. per μg) $\times 10^3$]	Confidence‡ interval ($P=0.05$)
57	Light grey	Coccoliths	12	47	904
28	Light beige	Coccoliths	15	52	1,409
21	Light grey	Coccoliths	22	23	1,005
11	Off white	Diatoms	41	135	1,604
14	Off white	Aragonite	62	200	1,704
30	Dark brown	Coccoliths	12	1700	60,023
73	Nearly black	Coccoliths	15	2400	90,012
13	Dark brown	Coccoliths	22	800	38,026
91	Dark brown	Diatoms	41	1200	10,024
65	Nearly black	Sapropel	62	3900	80,035

* Samples were coded to avoid unconscious bias of the counts.

† Mean granular densities per μg dry wt of sediment $\times 10^3$.

‡ Two standard deviations erected about the opaque granule densities were based on three replicates.

stubs and gold coated. Light- and dark-banded material from individual microlaminae was removed using fine dissecting forceps under a dissecting microscope to ensure that the material was not contaminated by adjacent lamina.

To determine the abundance (density) of dark coloured granules in the light and dark microlaminae, the previously isolated sediments were dried for 24 h at 80 °C and weighed after cooling. A sample of 0.1 mg from each of five light coloured lamina and five dark coloured lamina was suspended in 1.0 ml distilled water and a 0.1 ml aliquot of the homogenate was placed on an haemocytometer. The number of black granules were recorded for each of 40 randomly selected haemocytometer fields. A similar technique was used to determine the density of the photosynthetic bacteria from the IKI preserved water samples from the Black Sea.

Vertical distribution of photosynthetic bacteria in the Black Sea

As photosynthetic bacteria are light dependent, they develop population maxima at the minimal distance from the surface at which sulphide is available to them¹². In the Black Sea, sulphide is unavailable above a depth of 150 m at most locations¹⁴. Furthermore, these photosynthetic bacteria are killed by minute concentrations of oxygen¹⁵. The water column of the Black Sea is rarely anaerobic above a depth of 150 m (ref. 14).

On 31 October 1977 the photosynthetic bacteria were found concentrated at a depth of 200 m at the Kilyos station in the Black Sea (Table 1). Micrographs of these bacteria (Fig. 1a) indicated that the dominant form possessed an external morphology similar to that described by Buchanan and Gibbons¹⁵ for the photosynthetic bacterium *Thiocapsa roseopersicina* (spherical with a mean diameter of 1.5–2 µm). Many of our samples seem to contain blue-green algae as well as several other types of photosynthetic bacteria which we have yet to identify.

Individuals of *T. roseopersicina* in samples which were inadvertently exposed to small amounts of oxygen before preservation were often surrounded by a slime-filled envelope which may have caused them to aggregate into clumps (Fig. 1b). Irregularities in cell shape (Fig. 1a) were ascribed to the internal storage of elemental sulphur which this species produces photosynthetically as a waste product during the oxidation of H₂S^{12,15}.

Photosynthetic bacteria taken from the other end of the Black Sea near Trabzon, Turkey on 17 November 1977 were similar to those at Station B but were concentrated at a slightly shallower depth (160 compared with 200 m) (Table 1).

Comparing our autumn photosynthetic bacterial densities with the winter densities reported by Kriss¹⁶ (for his Yalta-Batumi stations) it was evident that his February photosynthetic bacteria maximum was located at a much greater depth than ours. By late February the water at Kriss's winter stations was oxygenated to a depth of 300 m and the photosynthetic bacteria were concentrated at a depth of 500 m, indicating that oxygen might have been entrained down to that depth some time earlier or alternatively that the high bacterial densities which he observed at 500 m and deeper, may have included large numbers of dead bacteria which were settling out. Kriss's winter observations suggested that Black Sea waters below 200 m might be seasonally exposed to dissolved oxygen.

Grasshoff calculated that 500 km³ of surface waters containing an estimated 5 × 10⁶ metric tons of oxygen penetrates the chemocline of the Black Sea each year¹⁴. Furthermore, Brewer *et al.*¹⁷ observed elemental sulphur as well as manganese and iron oxide granules in the anaerobic surficial sediments of the Black Sea along the Turkish (Anatolian) coast in 1,000 to 2,000 m of water. They interpreted this to mean that oxygen-rich surface waters had contacted the sulphide, manganese and iron-rich waters at and below the anaerobic chemocline in the Black Sea¹⁰ oxidising these waters and thereby precipitating elemental sulphur, iron and manganese oxides.¹⁷

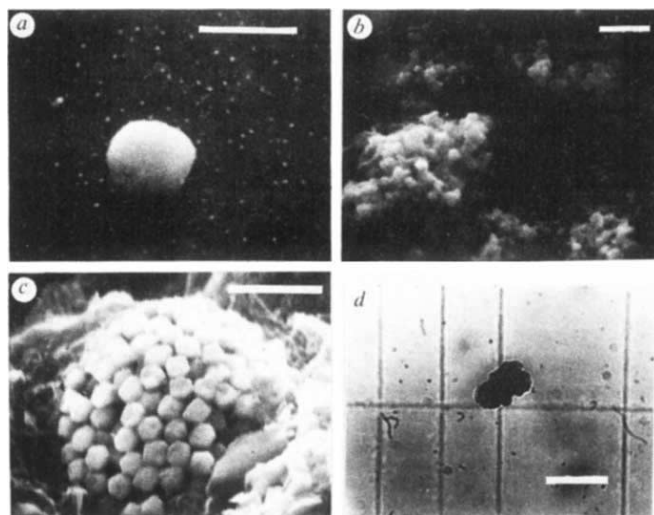


Fig. 1 a–c, SEM photographs. a, *Thiocapsa roseopersicina* from a depth of 160 m at the Trabzon, Turkey station. Irregularities in cell surface may be the result of the internal storage of elemental sulphur¹⁵. Background specks are crystals caused by the evaporation of Lugol's¹³ IKI preservative in which the original samples were stored. Scale bar, 2 µm. b, A colony of *T. roseopersicina* from 160 m at the Trabzon station which had been inadvertently exposed to dissolved oxygen before preservation. Cells are bound together by thick mucillagenous secretions. Scale bar, 10 µm. c, A pyrite (FeS₂) framoid from a black microlamina at 4 cm depth in a sediment core from the Glomar Challenger station 381 (ref. 5). Scale bar, 2 µm. d, A transmitted light microscope photograph of a pyrite granule from a depth of 260 m in the Black Sea near Kilyos, Turkey. Although its framoid structure was later verified using SEM, its possible resuspension cannot be ruled out. Scale bar, 5 µm.

Vertical mixing of oxygenated surface waters to depths of 300 m or more in the Black Sea seems to occur in winter when the surface waters have been cooled and the water column approaches homeothermy. In addition, increased surface salinity before late winter rains reduces the amplitude of the vertical salinity gradient¹⁸. It is not surprising, therefore, that Kriss¹⁶ observed dead photosynthetic bacteria in well oxygenated water above 100 m after heavy winter storm activity presumably swept them up from the chemocline.

These four lines of evidence from independent studies all indicate that a seasonal mass mortality of photosynthetic bacteria is the result of partial and temporary oxygenation of the upper anaerobic layers of the Black Sea in winter.

Figure 2 provides a schematic representation of these events. In the uppermost diagram, six zones are represented by the letters A–F. These symbols depict the following portions of the Black Sea's water column: (A) the warm-water upper mixolimnion; (B) the metalimnion, upper and lower boundaries of which are represented by the dashed lines; (C) the oxygenated cold-water portion of the mixolimnion; (D) the anaerobic portion of the mixolimnion; and (E) the permanently anaerobic monimolimnion. The 'dots' concentrated along the top of the anaerobic mixolimnion (zone D) represent the photosynthetic bacterial plate.

During winter (central diagram) the metalimnion disappears as the thermal gradient (not shown) becomes less intense as surface temperatures decrease¹⁸. Except for a relatively few photosynthetic bacteria (which secrete sufficient slime to form a bacterial mass (Fig. 1b) which is enough to prevent dissolved oxygen from reaching the core before the bacterial mass has settled into deeper anaerobic waters) the intrusion of oxygen into the previously anaerobic portion of the mixolimnion (zone D) results in the mass mortality of all the anaerobic bacteria in this zone. Subsequently, these dead bacteria settle towards the bottom of the Black Sea at a rate of between 1 and 2 m h⁻¹ (ref.

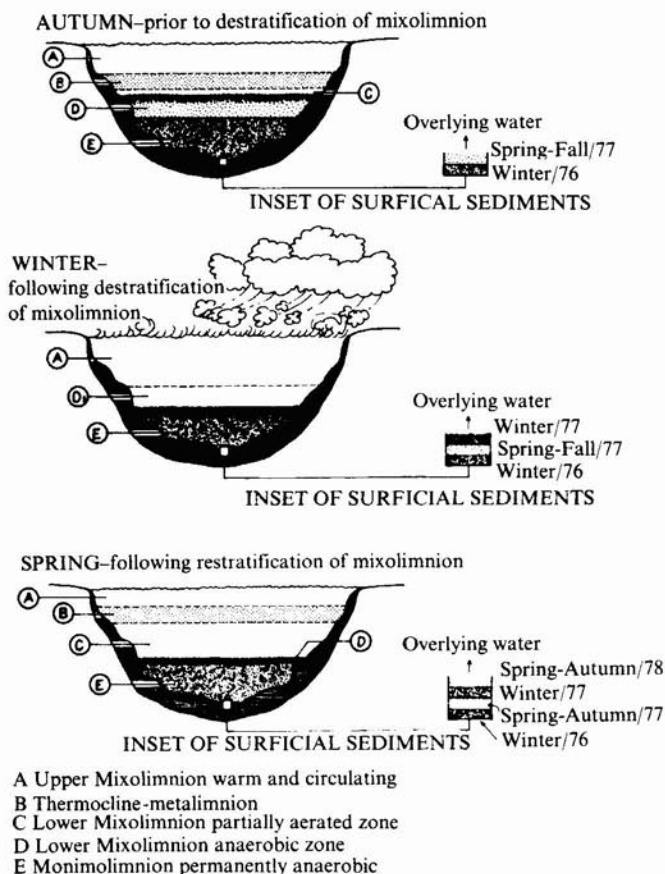


Fig. 2 Schematic representation of the major seasonal changes in the vertical distribution of planktonic photosynthetic bacteria in the Black Sea with insets depicting the formation of light and dark microlaminae (see text).

11). During their descent they serve as nuclei for the formation of black FeS-rich compounds. Many of these compounds later form raspberry shaped pyrites (FeS_2) called 'framboids' (Fig. 1c, d).

The bottom diagram in Fig. 2 depicts the water column of the Black Sea in early spring when the majority of the mixolimnion is still oxygen rich. As spring and summer pass, decaying organic matter (tripton) from the overlying water accumulates on or near the chemocline where its continued decomposition removes any dissolved oxygen from the overlying water mass. This process increases the thickness of the anaerobic region of the mixolimnion (zone D). This in turn permits the photosynthetic bacteria to move closer to the surface where light intensities are higher¹⁹. By early winter, most of the subthermocline mixolimnion has been deoxygenated and a condition similar to that depicted in the uppermost diagram of Fig. 2 exists. The process previously described then repeats itself if winds of sufficient intensity to mix oxygenated surface waters to the depth of the photosynthetic bacterial plate reoccur.

Dark lamina formation

Metal sulphides such as FeS are extremely insoluble in neutral or alkaline water such as that found in the Black Sea²⁰ ($\text{pH } 7.5 \pm 0.2$). Thus HS^- in the presence of ferrous ions will form FeS compounds which give anaerobic sediments their characteristic black colour²². However, the exact nature of the compounds that form is dependent on the presence and concentration of a number of additional species in solution such as carbonates, silicates and heavy metals²⁰.

In the conditions observed in the Black Sea (alkalinity 4.4 meq l^{-1} (ref. 21); H_2S (HS^-) concentration of $300\text{--}350 \mu\text{g atom l}^{-1}$ (ref. 21); a temperature 7 and 9°C ¹⁸; salinity of 22 ppt ¹⁰; and depending upon the depth of water, pressure of up to 200 atm) the precipitation of iron monosulfides would be

expected to occur at a ferrous iron concentration of roughly $4 \times 10^{-7} \text{ mol l}^{-1}$ (ref. 20). Only the sulphide-rich chemocline in the Black Sea contains such high ferrous iron concentrations¹⁴. Iron sulphide granules have been previously described for the sediments of the Black Sea^{5,22}. The general consensus seems to be that some of these black granules such as the pyrites may form during early diagenesis²³ however, Degens² described these same framboids in water samples taken several hundred metres above the sediment-water interphase in the Black Sea. These observations suggest that framboid formation begins in the water column following the mass mortality of photosynthetic bacteria. Since most of the Black Sea Basin is $1,000 \text{ m}$ or more in depth these bacteria would not reach the bottom for nearly 50 d given a sedimentation rate of $1\text{--}2 \text{ m h}^{-1}$. Thus it may be possible for their mineralisation and subsequent pyritisation to occur during their descent.

Data presented in Table 2 indicate that when black granule densities exceed about 1×10^6 per μg dry wt of sediment, a dark layer can be expected to form in the recent (coccolith-rich) sediments of the Black Sea.

The term black granule has been used above to indicate any opaque mass viewed with transmitted light microscopy. We are currently working (with S. Haynes) to determine the chemical composition of these iron- and sulphur-rich black granules. Several polished plastic impregnated cross-sections from the microlaminated Black Sea surficial sediments ($0\text{--}34 \text{ cm}$) have been produced. Pyrite framboid concentrations in these polished sections were 2 to 3 times higher in the dark coloured microlamina than in the light coloured ones.

Our studies and those of others^{8,16} have indicated a massive accumulation of photosynthetic bacteria just below the sulphide-rich chemocline in the Black Sea. These obligate anaerobic bacteria are instantly killed by the introduction of dissolved oxygen into their immediate environment.⁹ Following winter storm activity, large numbers of these photosynthetic bacteria have been observed in the surface waters of the Black Sea,¹⁶ indicating that vertical mixing had taken place.

The death of the photosynthetic bacteria on exposure to dissolved oxygen results in their gradual mineralisation as they sink slowly towards the bottom. During this process of mineralisation, these sulphur-filled bacteria act as nuclei for ferrous sulphide framboid formation. These framboids enter the bottom sediments of the Black Sea along with other sedimenting particles such as diatoms, coccoliths and clastics. Depending on the final black granule density and the degree of 'whiteness' of the underlying layer, a dark microlamina may form as shown inset in Fig. 2. However, if storm events during any particular winter fail to generate winds capable of oxygenating the chemocline, bacterial mass mortality there will fail to occur. As a result, a dark-coloured microlamina may fail to form. Discrepancies between 'varve' count and ^{14}C chronologies in recent Black Sea sediments may in part be related to this phenomenon.

The history of the oceans is in part the story of the progressive oxidation of their primordial anoxic environment²³. Degens and Stoffers⁵ have related the evolution of the oceans to the recent history of the present-day stratified waters of the Black Sea. The sediments of this curious body of water bear a striking resemblance to those deposited during Cambrian and Precambrian times in the bottom of some of the then existent world oceans⁵.

Data presented here indicate that dark lamina formation in this meromictic body of water is intimately associated with the mass mortality and subsequent mineralisation of the sulphur-rich photosynthetic bacteria which colonise the chemocline in the Black Sea.

We thank Drs Muzaffer Demir, Necla Demir, C. Karadeniz and A. Sengün for assistance, Dr Egon Degens (Hamburg University) and staff of the Doherty-Lamant Geology Laboratory for Glomar Challenger samples, and Howard Melville for help in electron microscopy.

Received 20 April; accepted 6 July 1978.

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letters to nature

High energy X-ray spectrum of Her X-1

OBSERVATIONS of Her X-1 taken during the on-state of its 35-d cycle¹ between 31 August and 12 September 1977 with the high-energy X-ray detector on board OSO 8 are reported here. A detailed analysis of the time-variable background spectrum suggests that the line features observed in our uncorrected time-averaged spectrum and those previously reported at 25, 60 and 135 keV can be caused by a $\sim 1\%$ change in detector gain. After correcting for this effect, a $3\text{-}\sigma$ upper limit of 4×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ is obtained on the line flux at 60 keV in the time-averaged spectrum, a flux significantly lower than that observed by Coe *et al.*² 6 months earlier. A $3\text{-}\sigma$ upper limit of 2×10^{-3} photon $\text{cm}^{-2} \text{s}^{-1}$ obtained for our pulsed spectrum at the same energy is higher than, and hence consistent with, the positive result reported by Trümper^{3,4} from a balloon flight during the same time interval.

The discovery by Trümper *et al.*^{5,6} of two strong lines in the pulsed X-ray spectrum of Her X-1 has inspired many other groups to search for this phenomenon. The stronger of the two lines, at 58 ± 5 keV, has now been observed by Trümper *et al.* on two separate balloon flights, one in May 1976⁵⁻⁷ and the second in September 1977^{3,4}. The second line, at an energy of approximately 110 keV, was observed in 1976 at the 2.2σ level, but in 1977 the line flux had decreased to below the detection sensitivity⁴. The measured absolute flux values for the two lines are given in Table 1. The credibility of these results is greatly enhanced by the observation that the flux in the lines pulses with the same 1.24-s period measured at lower energies. The time-averaged source spectrum can be distorted by systematic variations in the background spectrum or in the detector response. Because such variations are unlikely to occur with the same period as the observed pulsations from Her X-1, the use of the pulsed spectrum should eliminate these systematic effects.

No observation of Her X-1 by any other group has had the combination of sensitivity, energy resolution and time resolution necessary to positively detect and resolve either of these two lines. The reported line fluxes obtained by other groups at various different energies are given in Table 1. Unless otherwise indicated, the tabulated values are the line fluxes averaged over the 1.24-s period, or they are $3\text{-}\sigma$ upper limits. The standard deviation in the line fluxes are quoted directly from the respective authors and, in general, include the uncertainties in line width and instrument response function in addition to the statistical uncertainties. None of these reported line fluxes is

greater than 2.5σ although the statistical significance of the raw data of Coe *et al.*² is somewhat higher than this. Such a significance level is, however, reduced if the possibility of a line feature at any other energy is considered⁸. Recent results from the high-energy X-ray detector on HEAO 1 indicate a pulsed excess above the extrapolated low-energy spectrum consistent with the 1977 flux observed by Trümper *et al.* in the 58-keV line¹⁰.

The observations reported here were made with the high-energy X-ray detector on OSO 8, which is described in detail elsewhere¹¹. The instrument is an actively shielded CsI(Na) scintillation detector with a sensitive area of 27.5 cm^2 and a circular field of view with a full width at half maximum (FWHM) of 5° . The timing resolution is 0.3125 ms for each recorded photon, and the energy resolution is ~ 25 keV (FWHM) at

Fig. 1 The calculated counting rate spectrum of Her X-1 determined for the total observing time from 31 August to 12 September 1977. The counting rate is plotted on a linear scale to show the negative calculated fluxes at 45 keV, 70–100 keV, and >170 keV and also the apparent line features centred at ~ 60 keV and ~ 135 keV.

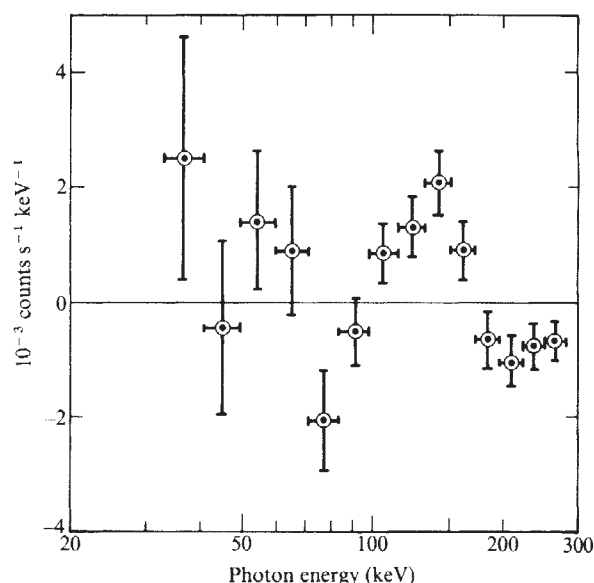


Table 1 Reported X-ray line fluxes from Her X-1 in photons $\text{cm}^{-2} \text{s}^{-1}$

Date	Vehicle	Refs	25 keV	60 keV	110 keV	135 keV
May-June 1972	OSO 7	8	$(2.4 \pm 1.2) \times 10^{-2}$	—	—	—
15 May 1975	Balloon	9	—	$< 6 \times 10^{-3}$	—	$< 4 \times 10^{-3}$
24 June 1975	Balloon	15	—	$< 2.6 \times 10^{-3}$	$< 1.9 \times 10^{-3}$	—
				Pulsed	Pulsed	
3 May 1976	Balloon	5, 6	—	$(2.9^{+3.0}_{-1.0}) \times 10^{-3}$	$(1.4 \pm 0.6) \times 10^{-3}$	—
				Pulsed	Pulsed	
3-13 Feb 1977	Ariel 5	2	—	$(1.7 \pm 0.7) \times 10^{-2}$	$(1.7 \pm 0.6) \times 10^{-3*}$	—
					70-280 keV	
3 Sept 1977	Balloon	4	—	$(1.1 \pm 0.1) \times 10^{-3}$	$< 10^{-3}$	—
				Pulsed		
				$< 9 \times 10^{-3}$	—	—
				Pulsed		
31 Aug- 12 Sept 1977	OSO 8 (proportional counter)	Pravdo (personal communication)	—	$< 4 \times 10^{-3}$	—	$< 2 \times 10^{-3}$
		This paper time-averaged	—	$< 2 \times 10^{-3}$	—	$< 1 \times 10^{-3}$
	OSO 8 Csl (Na)	This paper pulsed	—	Pulsed		Pulsed

* M. J. Coe, personal communication.

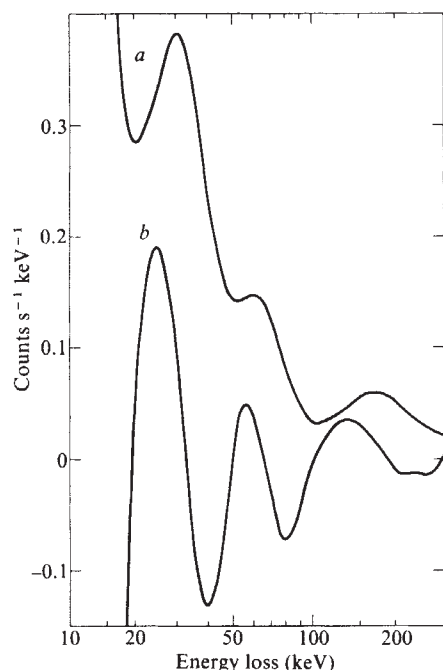
60 keV. The instrument is mounted in the rotating wheel section of OSO 8 with its field of view offset from the anti-spin axis by 5° . During the Her X-1 observations, the anti-spin axis of the spacecraft was orientated at an angle of $(5 \pm 0.5)^\circ$ from the direction to the source thus causing a modulation of the source signal for each wheel rotation. Our method of analysis uses this 10-s modulation to determine an accurate time-averaged source spectrum in the presence of the rather large and variable detector background¹². The pulsed spectrum is determined by combining the on-source data modulo the source period taken from Pravdo *et al.*¹³. The photon detection times are first adjusted for the time-of-flight variations between the source and the satellite.

The time-averaged spectrum obtained from all of our Her X-1 data shown in Fig. 1 contains two significant line features, one at ~ 60 keV and the other at ~ 135 keV. These features are not evident, however, in the pulsed spectrum. Furthermore, the

calculated source fluxes at 45 keV, 80 keV and > 180 keV were found to be negative. We now believe that the apparent line features and the negative source fluxes are all caused by a small change in the gain of the photomultipliers at each wheel rotation. Such a change in gain, of the order of 1% or less, can result from the changing orientation of the photomultipliers (RCA 31016F) in the Earth's magnetic field. A cylinder of 4-mil CO-NETIC magnetic shielding (Perfection Mica Co.) was put around each photomultiplier, but a residual variation of gain with magnetic field remains. This is presumably because it was not possible to extend the magnetic shield one diameter beyond the photocathode as recommended by RCA. The effect of such a change in gain on the detector counting rate depends on the shape of the background spectrum. Our background spectrum is shown in Fig. 2 together with the difference between two background spectra measured with a gain difference of 1%. Such a difference spectrum is the apparent source spectrum which would be calculated in the absence of any real source. The line features and the negative source strengths in the time-averaged Her X-1 spectrum clearly match this difference spectrum both in energy and in the relative amplitudes of the peaks and valleys. The possibility⁸ of a third positive feature at 25 keV is interesting although, in our measurements, such an excess is lost in the statistics of the real source flux at that energy.

Assuming that a small gain change accounts for the features in the source spectrum, it is easy to compute the true time-averaged spectrum. This is possible in spite of the complicated variations in the strength and direction of the Earth's magnetic field seen by the photomultipliers as the satellite moves in its orbit. The shift in background spectrum resulting from the gain change at each wheel rotation always results in an apparent source spectrum equal to the difference spectrum shown in Fig. 2 multiplied by some factor which can be either positive or negative. This is true only when the shape of the background spectrum remains constant. This is satisfied to the required accuracy using only data collected well out of the South Atlantic Anomaly. The true source spectrum is determined by subtracting the background difference spectrum, for some mean value of the gain change, from the calculated source spectrum. The mean value of the gain change can be most easily determined by a least-squares technique in which the assumed gain change is adjusted until the calculated source strengths in all energy bins are positive or zero to within the statistical uncertainties. The time-averaged spectrum obtained in this way and unfolded through the detector response function using the methods of Dolan *et al.*¹² is shown in Fig. 3. Only data from the non-eclipsed parts of the four binary periods during which the source flux was

Fig. 2 *a*, Detector background spectrum for the Her X-1 observing time. *b*, The difference ($\times 25$) between two background spectra measured with a gain difference of 1%.



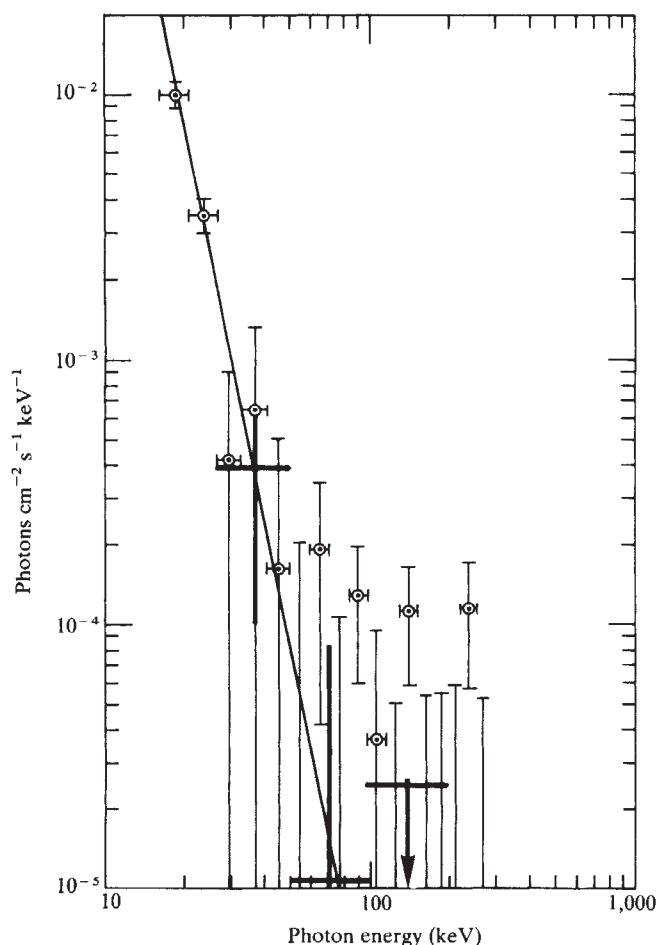
highest (31 August–6 September 1977) were used to obtain this spectrum. No significant features are evident above the 3σ levels given in Table 1. Positive detection of the pulsed flux was made up to an energy of 33 keV and no significant features are observed in the pulsed spectrum above the 3σ upper limits given in Table 1.

An understanding of features observed in our time-averaged spectrum of Her X-1 has been demonstrated in terms of small changes in gain of our X-ray detector. Such changes in gain can produce apparent line features in the spectrum at energies which have previously been reported in the literature. Brecher and Ulmer⁸ have pointed out the probable relationship to the detector background of the 1.5σ feature at 25 keV in the OSO 7 spectrum of Her X-1. Durouchoux *et al.*⁹ have shown that a small change in gain can explain the apparent line features at 57.5 and 137.5 keV in their time-averaged Her X-1 spectrum obtained on a balloon flight in May 1975. Coe *et al.*² report a line feature at 64 ± 6 keV in the time-averaged Her X-1 spectrum

Fig. 3 The time-averaged spectrum of Her X-1 for the period 31 August to 6 September 1977, during which the intensity was at its highest level. The count-rate spectrum for this period was corrected for an average change in gain of 0.7% and unfolded through the detection efficiency, K-escape probability, and detector resolution using the method of Dolan *et al.*¹² The best fit power law indicated is of the form

$$(2.4 \pm 0.4) \times 10^{-3} (E/25)^{-4.9 \pm 0.7} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}.$$

The circled points are for energy bins each the width of the detector resolution (1σ) at that energy. None of the points in the spectrum deviate from the power-law fit by more than 2σ . The heavy lines show the source flux averaged over the wider energy intervals indicated. The vertical lines indicate 1σ statistical uncertainties or 1σ upper limits.



from the high-energy X-ray detector on Ariel V. Attempts to explain their result in terms of a gain change have not been successful because the detector background spectrum does not have the required shape¹⁴.

The upper limits on the line features in our pulsed spectrum of Her X-1 are consistent with the results of Trümper *et al.*³⁻⁷. They do show that the line features are not significantly stronger when averaged over a full binary period as compared to the flux measured during a relatively short balloon flight. Finally, it must be emphasised that the reported line at 58 keV remains highly credible in view of its pulsed nature. Confirmation by different observers and measurements with improved energy resolution to determine the true line width remain highly desirable objectives.

We thank Roger Thomas, the OSO Project Scientist, for his efforts in making this long observation of Her X-1 possible, and Steve Pravdo for supplying us with the observational parameters of the pulsations. G.S.M. was supported by NASA grant NSG 5066 and E.P.C. by contract NAS5-24451.

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Received 22 May; accepted 25 July 1978.

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Physical reality of shot noise model for the short-term variability of Cyg X-1

TERRELL¹ pointed out that the rapid intensity variation of Cyg X-1, first discovered in Uhuru observations², was well simulated by shot noise, that is, random superposition of shot pulses of constant shape ($\sum h(t-t_i)$; t_i is a random variable). Since then this model has become widely accepted and used to summarise the observational data³⁻⁷ which suggest the physical process of overlapping bursts of X-ray emission from local hot spots in an accretion disk around a black hole. The variability characteristics of shot noise are roughly determined by two parameters⁸, shot rate λ and effective pulse length τ_{eff} of a single shot $h(t)$. Almost all the observational data so far

obtained seem to be consistent with shot noise having a pulse length of a fraction of a second—although shot rate varies from one observation to another—which is thought to support the validity of the model. This letter points out that shot noise is only an example—and a rather artificial one—of a random process which can explain the basic observed properties of the variation (its correlation time and magnitude) and why it does not need to be singled out among many types of possible stochastic processes. Therefore, physical interpretation of the observation strictly based on the shot noise parameters may be misleading.

First note that a stochastic time series $x(t)$ is described by a set of statistics—the first order one $\langle x(t) \rangle$ (average), the second order one $\langle x(t_i)x(t_j) \rangle$ (variance and autocorrelation), the third order one $\langle x(t_i)x(t_j)x(t_k) \rangle$ and so on. A major concern in shot noise analyses has been to estimate the two parameters τ_{eff} and λ from observational data. Note, however, that shot noise can be completely assimilated to observational data by adjusting $h(t)$ so that its autocorrelation equals that of the data. The shot rate λ is a scaling factor for the magnitude of variation. The shot noise generated this way gives a complete simulation of the data up to the second order statistics. In other words, for any given time series a shot noise can always be generated so that there is no way to distinguish them within the second order statistics.

Such an all-inclusive nature of shot noise obscures the physical meaning of the shot noise parameters, especially λ if τ_{eff} has model-independent meaning of a characteristic time scale. Its applicability to data does not prove the physical reality that X rays are indeed emitted in pulses with shot rate λ , without other additional supporting facts, observational or theoretical. Shot noise was originally introduced to explain and analyse the fluctuation of electric current in vacuum tubes, which is justified because it is essentially a quantum effect: with Cyg X-1 there is no such justification.

Sutherland *et al.*⁹ have discussed an extended shot noise model in which steady component was taken into account, introducing another parameter, 'shot noise fraction' f . This discussion shows that λ and f cannot be uniquely determined by the second order statistics, and, in fact, the third moment $\langle x^3(t) \rangle$ was used to estimate the parameters. The analysis not only involves the third moment whose estimation error can be rather large, but also depends on the assumed shape of $h(t)$.

The usefulness of such an analysis is examined in the following example. One of the most basic and physically important stochastic processes, is brownian motion, its simplest model is described by Langevin equation¹⁰,

$$\frac{du}{dt} = -\gamma u + R(t)/m$$

where u and m are velocity and mass of a brownian particle respectively, γ is coefficient of friction, and R is random force acting on the particle which can usually be considered to have a white spectrum (infinitesimal correlation time). The resulting stochastic time series $u(t)$ may be similar, in a generalised sense, to shot noise; fluctuations of u are generated by R and decay with $1/\gamma$, that is $h(t) = h \exp(-\gamma t)$ except that h in this case is not a constant but follows Maxwellian distribution law. It is possible to apply the extended shot noise model forcibly to $u(t)$, but the best fit parameters λ and f will hardly have any physical meaning, and indicate still less the collision rate with surrounding particles and 'shot fraction'. Should we try to apply the model introducing still higher moments to deduce the distribution parameters of h ? The physically relevant part in this example is the correlation time $1/\gamma$ of the fluctuation and the magnitude of variance $\langle u^2(t) \rangle$ which is equal to kT/m (equipartition law), and higher order statistics are of secondary importance.

As illustrated by the above example (and relaxation and fluctuation theories in statistical physics), physical understand-

ing of variation should be sought from the second order statistics, before getting too deeply involved in a rigid model based on *ad hoc* assumptions. Assuming shot noise with $h(t)$ and λ defines the complete set of statistics (or the probability density) which is practically beyond observational tests. Whether variation is shot noise or gaussian noise or any other type of fluctuation does not affect the second order statistics; physical discussion is possible without such a specification.

Finally, there are observational indications in Cyg X-1 which do not favour the simple shot-noise view of overlapping unit bursts. Ogawara *et al.*¹¹ noted the energy dependence of the variation of the rocket data in the 1.5–25 keV range and discussed the compatibility with the shot-noise view. Recently I carried out a balloon observation¹² of the short-term ($\sim$ s) variability in the hard (≥ 20 keV) X-ray region with a large-area detector, to obtain an integral, wide-band picture of the variation. These results, together with those of the former rocket observation¹¹, suggest that the variation is essentially spectral, keeping the total X-ray luminosity relatively constant, although a narrow band observation indicates intensity variation. A possible model of such a variation based on the inverse-Compton emission from a two-temperature disc around a black hole^{13,14} was suggested, which may exemplify a physical discussion noncommittal to the higher order statistics. The detail of the observation and discussion will be published elsewhere.

I thank Professors M. Oda, S. Miyamoto and Y. Ogawara for useful discussions.

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Received 23 May; accepted 23 June 1978.

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The spectrum of the supernova in MCG43223

A POSSIBLE SUPERNOVA in the galaxy MCG43223 was found by Gilmore¹ on 8 May 1978. The complex emission and absorption features of its spectrum which we have recorded are described below. Our findings confirm that this object is a supernova of Type V.

Gilmore reported that, at the time of discovery, the object was ~ 17.5 mag and that it was not visible on plates taken on 8 March and 8 April. Its presence was confirmed² by taking a plate of the galaxy on 25 May using the UK 1.2 m Schmidt telescope, Siding

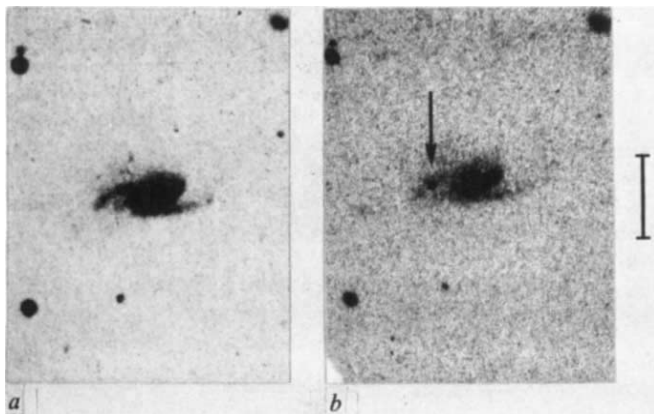


Fig. 1 Galaxy MCG43223: *a*, 60-min exposure on IIIaJ/GG395 filter 28 June 1976; *b*, 15-min exposure on IIIaJ/GG395 filter 25 May 1978. The scale is shown by the bar which is 20 arc s in length. North is at the top and east to the right.

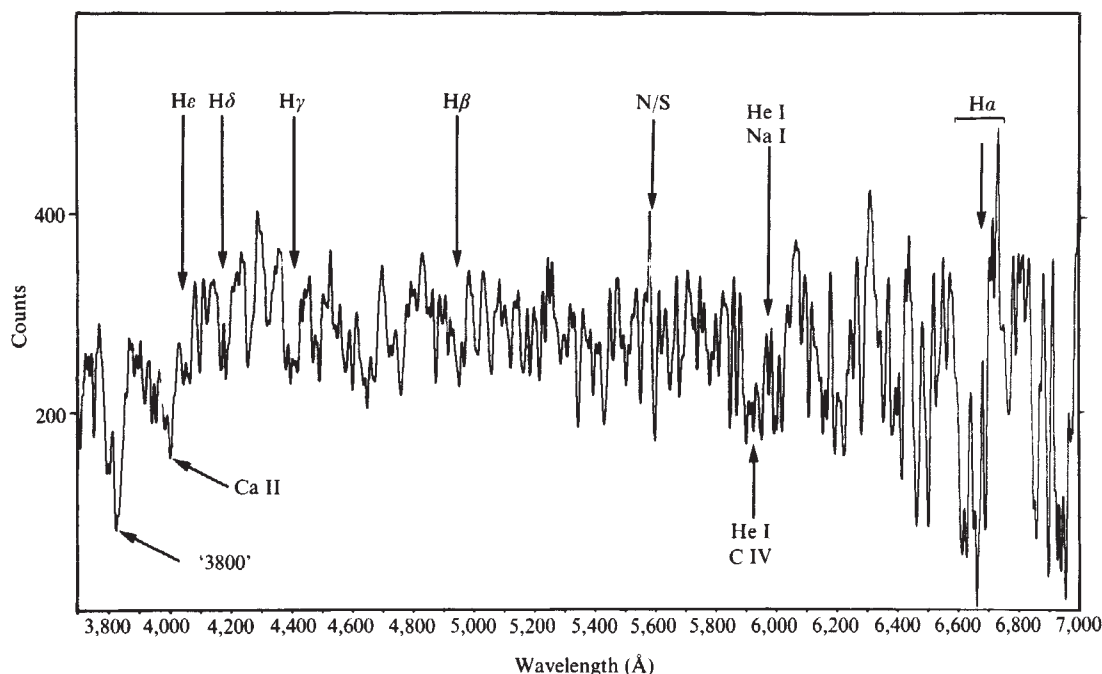


Fig. 2 The spectrum of the supernova in MCG43223: N/S refers to incomplete subtraction of the 5,577 Å night skyline.

Spring. The object's position was determined² to be RA = 13 h 27 min 32.60 s, dec = $-21^{\circ} 29' 24.0''$ (1950), being located 15 arc s due west of the galaxy's nucleus. Its photographic magnitude was estimated to be 20.0. A print taken from the UK Schmidt plate is shown in Fig. 1 where it is compared with a prediscovery photograph. Inspection of the print indicates that the object seems to be situated in a spiral arm of MCG43223. K.H.E. and J.C.B. obtained a spectrum of this object on 29 May with the Boller and Chivens spectrograph and Robinson Wampler³ image dissector scanner at the $f/15$ focus of the 3.9 m Anglo-Australian Telescope, Siding Spring. The spectrograph gave a nominal dispersion of 200 Å mm^{-1} , and a slit width of 2 arc s was used which corresponded to a final spectral resolution of $\sim 10 \text{ Å}$. The spectrum, which has been corrected for non-uniformity of the detector but not for its spectral response, is presented in Fig. 2. Bad weather severely curtailed our intended programme; we were unable to obtain standard star spectra or a spectrum of the galaxy.

We believe this spectrum, which shows a complex mixture of emission and absorption features, confirms that the object is a supernova in MCG43223. We have identified five features in the spectrum with H I, namely the Balmer series from H α through

to He; and we tentatively identify He I, C IV and Ca II. In addition the spectrum contains an absorption feature near $\lambda(\text{rest}) = 3,800 \text{ Å}$ which seems to be a common feature in supernovae spectra⁴.

Comparing our data with the spectra illustrated by Greenstein and Minkowski⁴, we identify the supernova as Type V (a subclass of Type II), as it shows many features in common with the Type II 1961 V in NGC1058⁴. This is the first time that a spectrum of a Type V has been recorded so close to maximum. However, there is some difficulty in making the comparison, as the published data for NGC1058 refer to a period ~ 173 d after maximum. For our data the spectrum must have been obtained between 21 and 50 d after maximum. A survey of plates taken of this field between 5 April 1978 and a month later should give a more exact timing of the maximum. The Balmer lines give $z = 0.012$. Equating this redshift with that of MCG43223 we obtain a distance of 65 Mpc, using $H = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$. With the estimated brightness of $V \sim 17$ we obtain $M_v = -17.1$ assuming no absorption, which is reasonable considering the high galactic latitude ($b = 40^{\circ}$). This absolute magnitude compares well with the accepted value for Type II supernovae,

$M_v = -17.54 \pm 0.26$ given by Tammann⁵. We intend carrying out further spectroscopy on the object, providing that it does not fade too quickly, to monitor spectral variations.

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Search for the 1.2 MeV γ -ray line of Walraven and Haymes

WALRAVEN AND HAYMES¹ have reported the possible detection of a ~ 1.16 MeV γ -ray line at a flux level of $(3.4 \pm 1.5) \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$ from a point in the galactic plane ($l = 345^\circ$, $b = 5^\circ$). If correct this could be an enormously important result constituting the first direct confirmation of theories of explosive nucleosynthesis (ref. 2 and refs therein). Clayton *et al.*^{3,4} have predicted that the most intense γ -ray line to be emitted from the radioactive debris of a typical supernova after ~ 3 yr will be the 1.156 MeV line from the decay of ^{44}Ti ($\tau_{1/2} = 48$ yr). However, the reported detection was at a marginal level of statistical significance (2.6σ). It was mainly the "remarkable double coincidence that just the theoretically-predicted spectral anomaly occurred" when the telescope was pointed at a region of the galactic plane believed to contain young supernova remnants, that encouraged these workers to report this result. Clearly an independent attempt to confirm this observation with a telescope of finer energy resolution is called for. Here we report the result of such an attempt. Our conclusion is that the reported positive detection was probably a statistical fluctuation.

A balloon-borne high-resolution (3.2 keV FWHM at 1.156 MeV) γ -ray telescope was flown on 11 November 1977 from Alice Springs, Australia. The instrument was essentially the same as that previously described^{5,6}, but an important modification made for this flight was the replacement of the central Ge(Li) detector with a large volume, $\sim 130 \text{ cm}^3$ high-purity germanium detector. This necessitated an increase of the effective entrance aperture in the 400 lb NaI anticoincidence shield to 16° FWHM at 1.156 MeV. The direction ($l = 345^\circ$, $b = 5^\circ$) was tracked for 1 h starting at 23.51 UT with the balloon at an atmospheric depth of 3.0 g cm^{-2} . The search for the 1.156 MeV line was terminated when our quick-look data revealed that no indication of the feature at the reported flux level was evident.

In Fig. 1 we show the sum of all our data in the vicinity of 1.16 MeV. Only corrections for drifts in the energy calibration with time have been made. No astrophysical γ -ray line is apparent in these data nor in any of our data within hundreds of keV of 1.156 MeV. These data can be used to set a 1.156 MeV γ -ray line flux limit. Using the known detector efficiency, instrumental dead time and atmospheric attenuation we find a

2σ limit of less than $2.8 \times 10^{-4} \sqrt{\text{width (in keV)}}$ photons $\text{cm}^{-2} \text{s}^{-1}$. Since the linewidth is expected to be ~ 40 keV (FWHM) (ref. 7) this corresponds to a 2σ limit of less than 1.8×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ which is a factor of two less than the reported intensity. Thus the reported positive detection seems likely to be a statistical fluctuation.

We thank P. E. Havey, G. C. Hauser, A. F. Hutters and J. J. Lochtefeld for technical support and the staff of the Australian Balloon Launching Service (led by P. Oates) and the US National Center for Atmospheric Research (led by H. Woody and R. Collett) who ensured a successful balloon flight.

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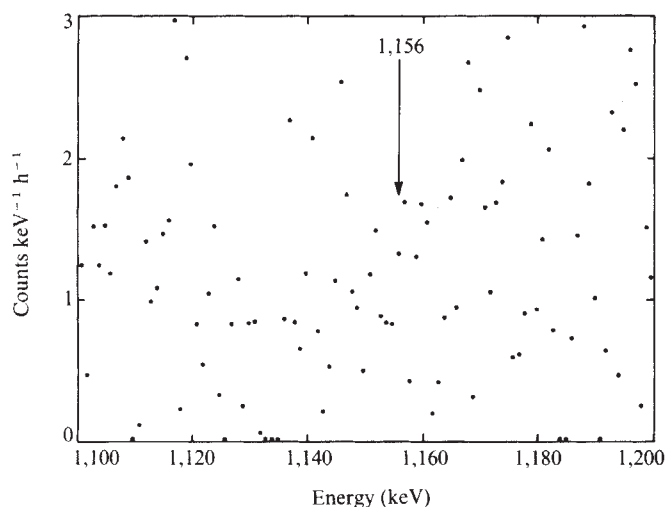
Does the troposphere respond to day-to-day changes in solar magnetic field?

WILCOX ET AL.¹ have reported a statistical correlation between the solar magnetic sector structure and the vorticity area index (VAI) during the period 1964–70. Characteristically, the VAI reached a minimum 1 day after the passage of a solar magnetic sector boundary (SSB), with the response being greatest in winter and at 500 and 300 mbar. This result has been the basis of many subsequent studies; in particular Wilcox *et al.*² strengthened their previous results by extending the period of analysis to the 11 year interval 1963–73. We have examined the period 1974–77 and find that this characteristic response is now no longer evident.

Williams has recently³ analysed the four components of the Lorenz energy cycle in an attempt to clarify the physical processes involved in the apparent solar modulation of the VAI. Although no statistically significant solar effects were discovered, some similarity was found in the behaviour of the eddy kinetic energy (KE) and the VAI. However, the KE exhibited a greater averaged response to the passage of an SSB (as obtained using a superposed epoch analysis) during the 1960s than the 1970s. Consequently, if there is a correlation between the KE and the VAI we might expect the effect of an SSB crossing on the VAI to also be weaker during the more recent years. This prompted us to extend the analysis to the period 1974–77 to determine whether or not the characteristic response of the VAI still existed during these years.

The VAI was calculated by the method described previously¹ using the National Meteorological Center data available at the National Center for Atmospheric Research. The VAI, at a particular pressure level, is defined to be that area of the Northern Hemisphere north of 20°N in which the absolute vorticity exceeds $24 \times 10^{-5} \text{ s}^{-1}$ plus that area in which it exceeds $20 \times 10^{-5} \text{ s}^{-1}$. This is a modification of the original index defined by Roberts and Olson⁴. In order to match the previous work, a superimposed epoch analysis was performed using the VAI and SSBs⁵ during the winter months 1 November–31 March. However, we required that all the VAI data be in this interval

Fig. 1 Energy spectra in the vicinity of 1.156 MeV. Raw data corrected only for drifts in the energy calibration. Each point represents a 1-keV wide energy bin.



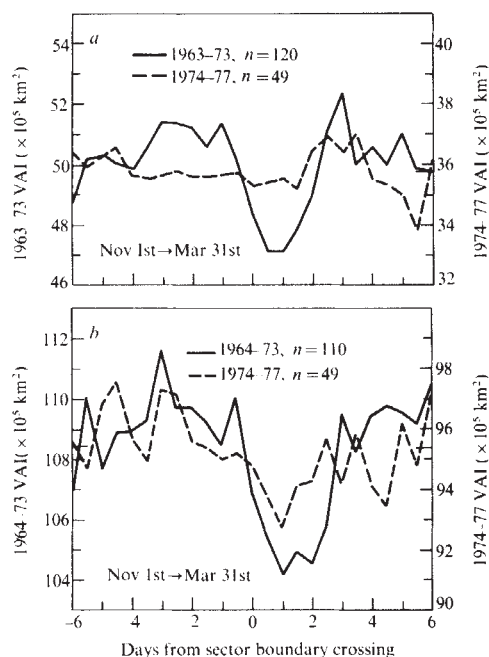


Fig. 1 Results of superposed epoch analysis of VAI plotted against SSBs during the periods indicated. *n*, no of SSB's included. *a*, 500 mbar; *b*, 300 mbar.

whereas the criterion used by Wilcox *et al.*¹ was that all the SSBs be in it. Our requirement eliminates 11 key days used by Wilcox² and 1 during the interval 1974-77. In all other aspects our approach is identical to that of Wilcox *et al.*¹.

Figure 1*a* illustrates the results at 500 mbar. For the interval 1963-73 we obtained results consistent with those of previous authors. This satisfied us that we were using the same analysis techniques as had been used in the earlier studies. However, for the years 1974-77 (our analysis ended in March 1977 so that 3½ years are included) we find a response completely unlike that of the previous years. Similarly, at 300 mbar Fig. 1*b* shows that although the absolute minimum is still on day +1 the magnitude of this response, at best, is greatly reduced. We thus find evidence to support the hypothesis of a correlation between the VAI and the KE.

In order to interpret this result we sought differences between the two time intervals which might qualitatively explain our findings. First, although the new result is for a short time interval, the number of key dates analysed, 49, is comparable to the 54 first analysed between 1964-70. Second, it may be argued that the years 1974-77 spanned a solar minimum so that we may expect little solar effect on the weather. This hypothesis was tested by examining a similar 3½ year period, 1963-66, which covered the previous solar minimum. However, during these years the VAI clearly exhibited the characteristic response to the passage of the SSBs². It therefore seems unlikely that the new result was caused by a lack of solar activity. Third, since a superimposed epoch analysis is essentially a cross correlation

integral, the results depend on any periodicities in either dataset. Thus, in this study, any differences in the spectrum of separation of key dates would influence the results. However, no obvious differences are evident in the histograms of sector widths as shown in Fig. 2. Fourth, it is clear from Fig. 1 that the typical values of the VAI are much lower during the more recent years. This suggests the possibility that the atmosphere is not in a 'suitable state' to respond to a solar impulse when the VAI is low. Finally, there is the possibility that the years 1963-73 or 1974-77 are yielding an anomalous result. Neither of these possibilities may be discounted using the current database.

As this is a purely statistical study there is no method for conclusively proving which, if any, of the last three possibilities is the correct interpretation. However, since there are, at present, no workable physical mechanisms, it seems that the simplest interpretation is that the years 1963-73 yielded an anomalous result which was of unusually high statistical significance⁶ and which was self-consistent for an unusually long time. We therefore conclude that our work weakens the evidence for this particular sun-weather correlation.

R.G.W. is grateful to the Fulbright-Hays Commission for financial support. This work was supported in part by Department of Energy contract ER-78-S-02-4634.A000, administered by the Aspen Institute for Humanistic Studies.

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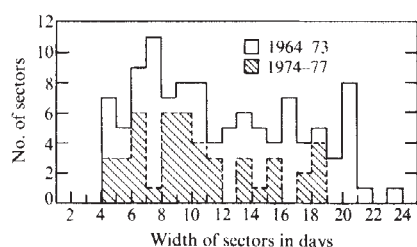
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Ordered arrangements of spheres of two different sizes in opal

GEM OPALS consist of a regularly ordered array of silica spheres of uniform size¹. Generally in any given gem stone the uniformity is maintained throughout the gem, although occasional samples contain bands in which the size varies from band to band. A sample of Brazilian gem opal of this banded type has now been found to contain a band in which there are spheres of two different sizes mixed together. Long-range ordering within this mixture has produced several different types of regular superstructures, which can be seen in section in electron micrographs of replicas of chips of this specimen (Fig. 1).

Packing arrangements of monodispersed spherical particles are of great general interest, and suspended latex spheres may order under the influence of electrostatic interaction from the surface double layers². Close-packed ordering also achieves a higher density than random packing, and it is generally conceded, but not proved, that a system of an infinite number of uniformly-sized spheres achieves its maximum density by regular close-packing³. The monodispersed spheres of silica in gem opal have been found to be packed in a close-packed manner⁴. Arrangements of mixtures of two sizes of sphere have been investigated experimentally⁵ and by computer simulation⁶ in order to determine packing densities, and to find out how the

Fig. 2 Histograms of width of sectors analysed in each period.



packing arrangement of one component is modified by the introduction of a second component of different size. No long-range ordering of the two components was observed in any of these cases, despite vibration of the arrays to simulate annealing⁵.

The regular arrangements of the two sizes of sphere in this unusual Brazilian sample were found in a number of replica fragments of a few chips from a single sample. The specimens used for electron microscopy were casually fractured, and so exposed surfaces of random orientation with respect to the array, with the orientation varying from fragment to fragment. The pieces of replica could not be related back to the original specimen. The appearance in an electron micrograph of a section through an array is expected to depend very much on the orientation of the section, but several different sections from this specimen could be recognised as coming from the same arrangement, and different ordered arrangements could be distinguished. Two approximate structures (to be described elsewhere) have been deduced from electron micrographs.

It appears that in this part of the opal there are just two sizes of sphere, with diameters of 0.18 and 0.1 μm respectively. In those places where ordering was observed it was most extensive, covering, for example, most of the area of a fragment of replica on a grid, for example, 1–2 mm. The different structural arrangements seem to be related to variations of the proportions of the two sizes of sphere in different places. Some places contained disordered mixtures of the two sizes, and in one place there was a boundary across which small spheres appeared to have penetrated into an array of larger ones. An example of part of the exposed surface of one of the ordered arrangements is shown in Fig. 1: it shows large spheres regularly dispersed among small ones, and close inspection shows that there is also a complex ordered arrangement of the small ones. How did the mixture of two sizes of spheres form and what was the driving force for long-range ordering?

It has been suggested⁷ that uniformity of sphere size in natural gem opal resulted from very slow, steady, near equilibrium conditions for the precipitation of silica from an underground solution. It is not clear what creates close-packing in the natural system. However, in laboratory experiments, monodispersed silica spheres settle quickly to a layer at the bottom of a vessel, and order (as shown by the formation of columnar grains exhibiting iridescence) increases over a period of months.

It seems likely that an understanding of the reasons for the ordering of the two sphere sizes found in the Brazilian sample might shed new light on the mechanism of opal formation. We have considered two possible mechanisms which could lead to ordering in the Brazilian sample. First, the two sizes of silica spheres might have formed concurrently and ordered whilst in

the colloidal state under the influence of long range double layer repulsions. This order could then be retained during sedimentation and compaction. Alternatively, ordering might have occurred solely under the influence of the Earth's gravitational field during compaction. This allows an alternative possibility of the two sphere sizes forming at different times and mixing by infiltration of the smaller spheres after an initial settling.

The first possibility is based on the knowledge that colloids consisting of spherical particles of equal size can order under the influence of repulsive double layer forces⁸. The question here arises as to what sort of ordering would be favoured by a colloid containing particles of two sizes. Would colloid theory predict a two-phase system with one phase containing small particles and the other phase containing large particles, or would a single mixed phase be preferred? A partial answer to this question has been obtained by considering pairwise potentials arising from van der Waals⁹ and double-layer forces¹⁰ between large spheres (U_{AA}), small spheres (U_{BB}), and mixed, large and small spheres (U_{AB}). For the simplest case of a system with equal numbers of small and large spheres we can simply regard the system as analogous to a binary alloy (see, for example, ref. 11). If then we find that $U_{AA} + U_{BB} > 2U_{AB}$, we conclude that a single mixed particle phase will result. Our calculations based on the observed sizes show that for systems with reasonably large double-layer interactions the above inequality is valid, down to a separation of the surfaces of the particles of about 50 Å, indicating that colloidal ordering could be responsible for the type of structures observed. As interparticle separations become less than 50 Å van der Waals forces become increasingly important and finally cause the particles to cohere strongly. At some later stage evaporation of the parent solution precipitates a final residue of silica which forms a cement and imparts additional strength to the opal. Gravity may play a part in compaction through sedimentation during opal formation in this model but is not essential to the ordering process.

The second ordering mechanism is one in which gravity is the controlling influence. One difficulty with the colloid model is in understanding how two quite distinct particle sizes could have been formed concurrently. In the gravity-controlled model we suppose that the smaller particles were formed after the larger ones, and penetrated into an already settled, but not yet compacted array of large spheres. Laboratory observations have confirmed that this process is possible for latex spheres¹². In these conditions sufficiently close contact would be maintained that pairwise interactions would be dominated by van der Waals forces. Our calculation shows that these forces alone do not favour the formation of a single phase, and therefore gravity must provide the ordering potential in this case. It follows that the stable system should be the one with the highest density. The density of the single phase would then be required to be higher than that of a close-packed monodispersed system, and this is certainly the case for one of the structures observed in Brazilian opal.

We conclude that it is not yet possible to determine which of the two suggested mechanisms of ordering occurs in the natural growth of opal. The fact that both major driving forces lead to a preferred AB (single phase) structure means that we are unable to use the two sphere size regions of the Brazilian gem to distinguish between the mechanisms. In nature both mechanisms may in fact operate. Their relative influences will depend on the precise physical and chemical characteristics of the parent solution, for example density, pH and ionic concentration.

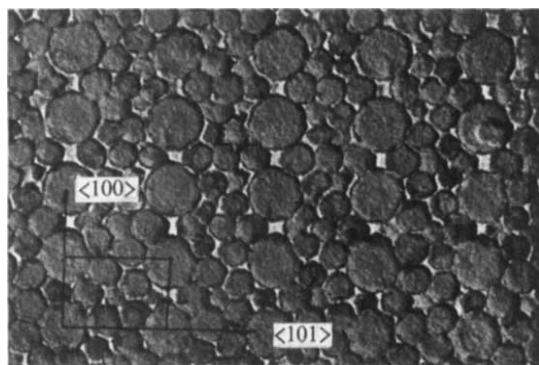
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Fig. 1 A small area of extensively ordered array of spheres of silica of two different sizes. A unit cell of surface structure is marked. The axes have been determined by relating this micrograph to sections found in other micrographs. The arrangement of small spheres varies slightly across the field of view because the section is slightly inclined to an axis.



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Anisotropy of melting for cubic metals

KINETIC studies of superheating of metals^{1,2} have shown that surfaces play an important part in initiating melting. Recent work on the anisotropy of vapour pressure³ suggests a strong dependence of melting on crystallographic orientation. There are models of melting⁴⁻⁷ which indicate that surfaces may become disordered (that is, lose their long-range order) at temperatures below the bulk-melting point. These temperatures are characteristics of the crystallographic planes of a metal. Based on simple considerations, we attempt here to estimate the melting temperatures of major low-index planes for close-packed cubic metals. The results are discussed in terms of low-energy electron diffraction (LEED) study and current theories of melting.

On the basis of Lindemann's melting criterion⁵, one can express in terms of harmonic approximation the relation between Debye temperature θ_D , melting point T_M , atomic weight M and mean-square amplitude of atomic vibration $\langle u^2 \rangle$ as⁸

$$\theta_D^2 = C \langle u^2 \rangle^{-1} [T_M/M] \quad (1)$$

where C , a constant, is equal to $9\hbar^2/k_B$ with $\hbar$ denoting the reduced Planck's constant and k_B the Boltzmann's constant. It has been well established through both experimental measurements and theoretical calculations that $\langle u^2 \rangle$ is much larger at a surface than in the bulk⁹. Consequently, the degree of anharmonicity should be much greater at the surface than in the bulk. From the various methods of studying surface anharmonicity¹⁰, the simple force constant method, which is based on anharmonic perturbation theory, is used here. Only the normal component of mean-square displacement denoted by $\langle u_\perp^2 \rangle$ is considered, as it is this parameter which is generally greater than the tangential component. The ratio of this value for surface (S) and bulk (B) that is, $\langle u_\perp^2 \rangle_S / \langle u_\perp^2 \rangle_B$ for various crystallographic planes, is only known for close-packed cubic metals as 1.87, 1.83 and 1.86 for (100), (110) and (111) planes, respectively¹¹. An analysis of equation (1) in terms of surface and bulk components followed by rearrangement would give

$$T_{MS}(hkl) = [\langle u_\perp^2 \rangle_S / \langle u_\perp^2 \rangle_B] [\theta_{DS}(hkl) / \theta_{DB}]^2 T_{MB} \quad (2)$$

A subsequent estimation of $T_{MS}(hkl)$ for planes (hkl) is presented in Table 1 for metals for which the various parameters of equation (2) are known¹²⁻¹⁴.

The present results on $T_{MS}(hkl)$ are considered to be fairly reasonable. For example, the surface melting temperature for Pb, which varies from (111) to (110) planes over a range of 49 K, is comparable with the 40 K obtained from LEED studies⁷. It is, of course, evident from equation (2) that T_{MS} values would depend on the choice of the ratio $\langle u_\perp^2 \rangle_S / \langle u_\perp^2 \rangle_B$ and on the adequate calculation of surface Debye temperature $\theta_{DS}(hkl)$ which is primarily based on summation of Morse potentials¹².

It has been pointed out¹⁵ that Lindemann's melting hypothesis consistently predicts the absolute melting temperature too high. It is argued in terms of amplitude of vibration of surface atoms that the 'critical amplitude' (for melting) will be reached at the surface before the interior. This view has been confirmed by LEED results⁷ and molecular dynamics studies⁴. The results in Table 1, with T_{MB} being consistently higher than T_{MS} , therefore agree with the above concept. It is also evident from Table 1 that T_{MS} decreases from (111) to (110) planes. Such a trend

Table 1 Some thermal properties of close-packed cubic metals

Metal	Bulk component		Surface component					
	Debye temperature* θ_{DB} (K)	Melting point† T_{MB} (K)	Debye temperature‡ θ_{DS} (K)			Melting point§ T_{MS} (K)		
			(110)	(100)	(111)	(110)	(100)	(111)
Ag	226.5	1,233	142	142	147	887	906	966
Cu	334.4	1,356	191	192	196	809	836	866
Ni	476.0	1,728	225	225	230	706	722	750
Pb	105.3	600	54	56	58	289	317	338
Pt	238.4	2,042	167	166	174	1,837	1,851	2,023

*Ref. 13. †Ref. 14. ‡Ref. 12.

§Estimated using equation (2).

apparently indicates the dependence of melting point on the density of packing of atoms in a particular crystal plane. One can thus conclude that a maximum vibrational instability is associated with the least densely packed plane. This conclusion is consistent with the theories of melting^{5,16,17}. Following the work of Maradudin *et al.*⁹, one would expect the amplitude of atomic vibrations to be maximal for planes with the least atomic packing density. It is known that the Debye temperature is closely related to the amplitude of vibration. As a result, θ_{DS} is expected to be lower with less closely packed planes; this is apparent in Jackson's results¹². Because, according to Lindemann's criterion, the melting point of a metal is directly proportional to the Debye temperature (equation 1), the lowest melting point is expected for a plane with the lowest surface Debye temperature, that is, with least close packing of atoms, as indeed has been indicated in the present work. On the basis of the estimated values of Table 1, a satisfactory quantitative relation has been obtained between $T_{MS}(hkl)$ and work function of the single crystal faces of a bare metal possessing close-packed cubic structure¹⁸.

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Superheated liquids containing suspended particles

THE superheat temperature limits of particle-free liquids have been previously investigated, notable by Skripov¹, Blander *et al.*², Apfel³ and Porteous⁴. Examinations of the homogeneous nucleation of liquid pentane have found the experimental limit of superheat temperature to be 146.5°C, which agrees well with the theoretical limit of superheat of 147.7°C calculated by Blander *et al.*² using the theories of Döring⁵, Volmer⁶ and Kagan⁷. Studies of superheating pure liquids have stressed the

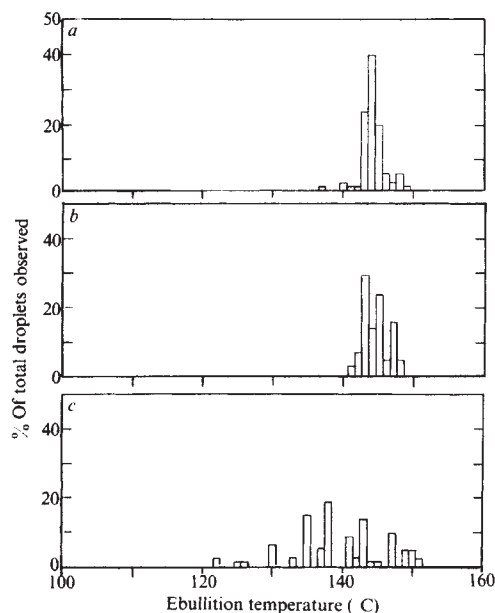


Fig. 1 *a*, Alumina (0.06 μm), run 3-15A; *b*, sericite (50 μm), run 3-14AC; *c*, carbon black (0.47 μm), run 6-12A. Particles suspended in pentane.

importance of keeping the liquid phase scrupulously clean and noted that the presence of 'motes' and particles interfered with obtaining experimentally consistent results because these served as nucleation centres; however, we know of no previous explicit study on the effects of suspended particles. We now report that liquid pentane and pentane/chloroform mixtures containing suspended solid hydrophobic and hydrophilic particles (carbon black, alumina, sericite) have been superheated as high as 110°C above their normal boiling points, and to within 3°C of the theoretical homogeneous limit of superheat of the particle-free liquid. This is the highest degree of superheat ever reported for liquids in the presence of solids other than smooth glass capillaries.

We examined the superheating of liquid *n*-pentane containing various amounts of suspended carbon black (Cabot; MT-X25, 0.47 μm diameter, Sterling R, 0.075 μm), alumina (Rhode Island Abrasives, 0.05 μm) and the mineral sericite

$[(\text{OH})_2\text{KAl}_2(\text{AlSi}_3\text{O}_{10})]$ (50 μm). We used a method of superheating liquids modified from that of Defour⁸, who superheated droplets of test liquid suspended in an immiscible, less volatile, host liquid, that acted as a 'liquid skinned' vessel, thereby avoiding contact with a solid container wall. We used a thermal gradient column modelled after those used by Moore⁹, Skripov¹⁰, Apfel³, Blander *et al.*² and Porteous⁴.

Pentane droplets (~1 mm diameter) were heated above their normal boiling point (36.1 °C) as they ascended slowly in a 47-mm i.d. glass column filled with ethylene glycol (host liquid), in which a vertical temperature gradient existed. The gradient section of the column was heated electrically with a spiral winding of nichrome wire. The spiral pitch decreased from 2 cm at the lower end of the column to 0.5 cm near the top. The temperature gradient ranged over 4.0–1.5 °C cm⁻¹ in the zone of superheat nucleations. The lower section of the column was unheated to permit calculation of the droplet diameter from its rate of rise. Temperatures at which nucleation occurred (explosively) were measured with thermocouples immersed in the host liquid at the level of nucleation.

Solid particles were suspended in the pentane by stirring. The suspensions were unstable and were stirred immediately before use. Pentane/chloroform solutions were also used because the polar chloroform improved the stability of the suspensions of carbon black. We found that superheat limits of the particle-free pentane/chloroform solutions were in approximate agreement with the molar average superheat limits of the pure components¹¹, and that the effect of carbon particles on the superheat limit of the mixed solvent was generally less than on the pure solvent. The chloroform not only stabilised the carbon but inhibited its nucleation propensity.

Our primary results are summarised in Table 1 and Fig. 1; the latter shows histograms of the percentage of total nucleation events against temperature. The nucleation temperatures for alumina (Fig. 1*a*) and sericite suspensions (Fig. 1*b*) are narrowly distributed, with means at 144.1 °C and 145.4 °C, respectively. The superheats attained with carbon black suspensions are lower (139.4 °C) and more broadly distributed, probably because of adsorption of gas on the carbon particles. Figure 1*c* is a histogram for a carbon black/pentane suspension (run 6-12A) which was refluxed for 18 h to drive off entrapped gas before injection into the superheating column. The deviation of nucleation temperatures is narrower, and the mean temperature of nucleation higher than for carbon suspensions with shorter pretreatment times.

Table 1 Superheating of suspensions

	Particle	Run no.	Soak time	Soak temperature (°C)	Mean particle diameter (μm)	Bulk volume % solids in test droplet	No. of observations	Mean ebullition temperature (°C)	Standard deviation
50/50 vol. % pentane/chloroform	None	4-3F	—	—	—	—	34	156.4	3.07
	Carbon black (Sterling R)	4-6AG	1 h	24	0.075	0.6	28	155.1	2.69
	Carbon black (MT-X25)	4-3E	1 h	24	0.47	4.0	32	153.8	2.20
	Carbon black (MT-X25)	4-4DI	1 h	24	0.47	6.0	32	150.5	3.81
100 vol. % pentane	None	3-6A	—	—	—	—	69	146.5	0.96
	Sericite	3-14AC	1 h	24	50.2	14.0	72	145.4	1.76
	Alumina	3-15A	1 h	24	0.06	12.0	108	144.1	1.57
	Carbon black (MT-X25)	3-15B	1 min	24	0.47	10.0	41	110.2	10.1
		3-11E	15 min	24			121	126.5	10.7
		3-11D	1 h	24			136	131.4	8.4
		3-11C	3 h	24			94	132.4	8.82
		2-28	1 h	31.6			143	133.0	4.50
		6-12A	18 h	31.6			80	139.4	6.53

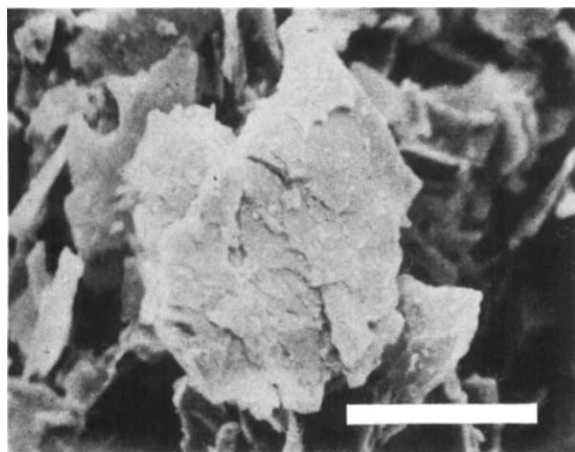


Fig. 2 Electron micrograph of sericite particle. Scale bar = 40 μm .

Carbon black particles are hydrophobic, whereas the alumina and sericite are hydrophilic. The consequent different wettabilities do not explain the observed superheat limit differences, that is, that the hydrophilic particles show a higher limit than the hydrophobic.

In general, the results are consistent with the nucleation theory of Volmer⁶, as extended by Cole¹², Jarvis¹³ and Eberhart¹⁴. The theoretical effect of interfacial energies on the limit of superheat would be that a change in wetting angle from 0° to $>90^\circ$ changes the calculated limits of superheat by only 2–3 $^\circ\text{C}$. The difference in the superheat limits observed for the carbon suspensions and the inorganic suspensions must therefore reside in the gas adsorption capacity of the former.

In spite of the agreement with nucleation theory, the results may seem counter-intuitive because we tend to think of a particle as a discontinuity in the liquid phase and, hence, a point where phase separation is likely to occur. The careful filtration and cleaning of fluids by other investigators would seem to have had little effect on their experimental results other than removing particles that contained entrapped gas.

Previous studies of superheated liquids in contact with solid surfaces other than freshly drawn glass or silica capillaries^{15–18} failed to produce superheats of pentane in excess of 10 $^\circ\text{C}$, whereas this study produced superheats of 74–110 $^\circ\text{C}$. That our surfaces are not smooth is evident from the electron micrograph shown in Fig. 2. Nevertheless, our liquid–solid interfaces suffer the initiation of a vapour phase about as reluctantly as does homogeneous liquid.

We thank Professor Lloyd Trefethen for bringing ref. 8 to our attention. This work was supported by NSF grant ENG-74-19478.

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The problem of the thrown string

ULAM¹ reports that “there was another problem which Fermi wanted to study but which we somehow never came to formulate well or to work on. He said one day, ‘it would be interesting to do something purely kinematical. Imagine a chain consisting of very many links, rigid, but free to rotate around each other. It would be curious to see what shapes the chain would assume when it was thrown on a table, by studying purely the effects of the initial energy and constraints, no forces’.” Sygne² posed the problem of estimating the average $\bar{D}$ of the rectilinear distance D between the ends of a flexible string of length L when thrown down at random on a table. Later³ he published conceptual models and some experiments which together increased the challenge (recently restated by Kingman⁴): the models yielded values of $\bar{D}/L$ equal either to 0 or 1 unity, whereas in three experimental series by different throwers of different strings, the values obtained were 0.32 (100 throws), 0.34 (50 throws) and 0.38 (60 throws). (Only for the last of these was the value of $\bar{D}^2/L^2$ also recorded, at 0.18.) Sygne wrote³: “the agreement of the results is rather striking and one might hope to explain the numerical value (say 1/3) on theoretical grounds . . . I hoped that someone would suggest a way of treating directly a system with an infinite number of degrees of freedom, but perhaps no such way exists”. Here we propose a solution along the desired lines by kinematical means.

Consider first the thrown string before it touches the table. Supposing that the rectilinear distance between its ends is x , how are the configurations of the string constrained by its continuity and its length? No element of the string can lie outside the ellipsoid having semi-axes $\frac{1}{2}L$, $\frac{1}{2}(L^2 - x^2)^{1/2}$, $\frac{1}{2}(L^2 - x^2)^{1/2}$, with the ends of the string at its foci; and any point within the ellipsoid may be intersected by the string. Thus the continuous nature of the string associates the accessible volume $\pi L(L^2 - x^2)/6$ with each value of x . We make this feature of the problem central to our treatment, and postulate that the probability density of x is proportional to the associated accessible volume. According to this postulate, if a large number N of throws is made, the number of cases for which the separation distance lies in the interval $(x, x + dx)$ is $3N(L^2 - x^2)dx/2L^3$, the proportionality factor being fixed by normalisation.

Next we introduce the vector $\mathbf{x}$ (of length x) from the lower to the higher end of the string, at the moment of impact of the string with the table, and model the effect on the separation distance of the collapse of the string onto the (horizontal) table, by projecting $\mathbf{x}$ on to the plane. Let θ be the smaller angle between $\mathbf{x}$ and the vertical. Assuming that the throws do not generate preferred values of θ , we adopt an isotropic distribution of (upwards) $\mathbf{x}$ -directions, and seek the distribution of projected lengths $D = x \sin \theta$. Consider the hemispherical surface of radius x lying flat on the table: its element belonging to the interval $(\theta, \theta + d\theta)$ contains the fraction $2\pi(x \sin \theta)(x d\theta)/2\pi x^2 (= \sin \theta d\theta)$ of the vertices of the vectors $\mathbf{x}$ drawn from the centre.

Then the number out of N throws for which, at impact with the table, the separation distance lies in $(x, x + dx)$ and the orientation angle in $(\theta, \theta + d\theta)$, is given by

$$n(x, \theta) dx d\theta = \frac{3N(L^2 - x^2)}{2L^3} \sin \theta d\theta dx \quad (1)$$

To obtain the observed distribution of the projections $D = x \sin \theta$, we change the independent variables x, θ in equation (1)

to x , D (Jacobian $(x^2 - D^2)^{-1/2}$), and integrate over x :

$$n(D) dD = \frac{3ND}{2L^3} \left[\int_D^L (L^2/x - x)(x^2 - D^2)^{-1/2} dx \right] dD \quad (2)$$

$$= \left[\frac{3N}{2L} \arccos(D/L) - \frac{3ND}{2L^3} (L^2 - D^2)^{1/2} \right] dD$$

Hence the probability density $\omega(\lambda)$ for the ratio $\lambda = D/L$ is

$$\omega(\lambda) = \frac{3}{2} [\arccos \lambda - \lambda(1 - \lambda^2)^{1/2}] \quad (3)$$

and for the density $\nu(u)$ of $u = D^2/L^2$ we find

$$\nu(u) = \frac{\omega(u^{1/2})}{2u^{1/2}}$$

Both ω and ν are convex and monotonically decreasing in the interval $(0, 1)$. While ω falls with only slight curvature from $3\pi/4$ to zero, ν falls from infinity to zero.

From equation (3) we obtain by straightforward integration the numerical values of the observables: $\bar{\lambda} = \bar{D}/L = 3\pi/32 \approx 0.29$, $\bar{\lambda}^2 = \bar{D}^2/L^2 = 2/15 \approx 0.13$. Considering the simplicity of our probabilistic postulate, these values compare favourably with the observations quoted above. At the conventional 0.05 significance level, there is no significant difference between the theoretical variance 0.046 and the observed variance 0.036, nor between the theoretical mean and the observed means in two of the experimental series. However, the differences of the theoretical mean from the largest observed mean, and from the mean of the pooled observations, are significant.

If the string was thrown so as to keep it in a plane parallel to the table throughout, the counterpart of ω would be constructed by replacing the ellipsoid by an ellipse, with the results $\bar{D}/L = 4/3\pi \approx 0.42$, $\bar{D}^2/L^2 = 0.25$. We note that all the observed means lie between those calculated from the ellipsoid and from the ellipse, and the observed mean square is correspondingly bracketed. This may be because (some of) the throws were made near the table and along it.

How does the above analysis apply in the case of a segment of length L belonging to a longer string? In this case our original postulate might require non-trivial modification to take account of the presence of extraneous bodies, namely the extra portions of string. The problem of the thrown string has points of contact with properties of coiling macromolecules in solution, especially when approached as a limit of a chain^{3,5}. However, systematic results for the former problem depend on persisting effects of the randomisation of initial conditions, whereas for the coiling molecules results depend on the more special state of thermodynamic equilibrium, independent of initial conditions.

We thank Professor J. F. C. Kingman and G. B. Price for interesting discussions.

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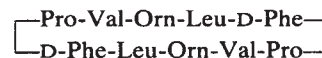
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The crystal structure of a hydrated gramicidin S-urea complex

THE cyclic decapeptide antibiotic gramicidin S has the sequence



Since its isolation by Gause and Brazhnikova¹, several authors have proposed possible conformations²⁻¹⁰. Of these models, the one that has gained most general acceptance is that of Hodgkin and Oughton², who proposed that the structure consisted of two β -chains linked by proline residues and stabilised by four intramolecular hydrogen bonds. Several derivatives of gramicidin S have been crystallised^{8,11}, but it had not been possible to solve any of the structures¹². We now report the structure of a gramicidin S-urea complex in which the gramicidin molecule has a conformation somewhat distorted from the Hodgkin-Oughton model.

In the presence of urea and HCl, gramicidin S crystallises as yellow elongated prisms from a solution in 95% ethanol. Crystal data: trigonal, $a = 25.8$, $c = 21.49$ Å, space group P3₁21, $D_m = 1.10$, $D_c = 1.06$ (based on $C_{60}N_{12}O_{10}H_{92} \cdot \frac{1}{2}[(NH_2)_2CO] \cdot 8H_2O$, $Z = 6$). Data collected on a Hilger and Watts Y290 4-circle diffractometer consisted of 5,695 observed reflections out of 6,353 measured. Because of crystal instability it was necessary to use several crystals during data collection.

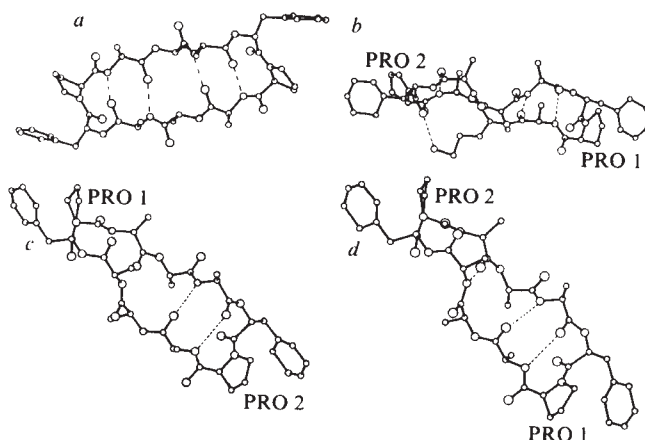


Fig. 1 Most of the side chains are omitted from the diagrams for the sake of clarity. *a*, A view of the molecule showing the four inter-chain hydrogen bonds, and the essential similarity of the structure to that proposed by Hodgkin and Oughton. *b*, A view showing the twist in the molecular backbone, and the unpredicted hydrogen bond involving the ornithine side chain. *c*, *d*, Two views related to each other by a rotation of approximately 180°, showing that the molecular backbone has approximate 2-fold symmetry.

The structure was solved by use of the multiple-solution direct methods program MULTAN¹³ developed in this laboratory. 100 phase sets were generated for 350 reflections ($E_{\min} = 1.86$), using 4,897 triple-phase invariants. The starting set consisted of one origin-defining and six other reflections (one with restricted phase and five with general phases). The phase set with the greatest combined figure of merit corresponded to an E -map which contained a 58-atom molecular fragment, and subsequent weighted E -maps revealed the remainder of the molecule and one half-molecule of urea.

The atomic coordinates and isotropic temperature factors were refined by the diagonal least-squares method, using fast Fourier transform techniques to generate structure factors and

gradients¹⁴. Refinement based on the 4,902 observed reflections within the sphere of 1.0 Å resolution gave an *R*-factor of 0.188, with approximate estimated standard deviations for the positions of carbon atoms within the main chain of 0.04 Å. The isotropic temperature factors (*B*) ranged from 5.0 to 25.1 Å². Because of the high thermal motion and the limited quality of the data, there is some uncertainty in the positions but not the presence of some of the side-chain atoms.

The molecular structure is basically similar to that of the model proposed by Hodgkin and Oughton². Figure 1a shows the four hydrogen bonds predicted from the model. The molecular backbone, however, is twisted from that of the model in such a way as to retain the approximate 2-fold symmetry but so that the hydrogen bonds between the two halves of the molecule are no longer parallel (Fig. 1b–d). In addition to these four intermolecular hydrogen bonds, there is an intramolecular hydrogen bond (N–O distance –2.88 Å) between one of the ornithine side-chain nitrogen atoms and the D-phenylalanine carbonyl oxygen atom which had not been predicted. The other ornithine nitrogen atom cannot take part in such a hydrogen bond but has a close, potentially hydrogen-bonded, contact to a water molecule.

The crystal packing is apparently stabilised by many hydrogen-bonding interactions. For each gramicidin molecule there is one half-molecule of urea (occupying a site on a crystallographic 2-fold axis) and eight molecules of water (six in general positions, two on 2-fold axes and one disordered between two sites approximately 1 Å apart). All these small molecules are within hydrogen-bonding distance, either of the gramicidin molecule or of each other. In addition to the possible intermolecular hydrogen bonding involving the small molecules, there is a hydrogen bond between atoms in the backbone of two gramicidin molecules related by a 2-fold axis (N–O distance –2.73 Å). All the polar atoms in the gramicidin molecule are involved in short, probably hydrogen-bonding, contacts of one of the types mentioned above, as are all the solvent molecules.

We are continuing our efforts to obtain more accurate data for the structure.

We thank the SRC for its support of this investigation; R.K. thanks the British Council for the award of a Research Fellowship.

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Thermoluminescence dating of sediment baked by lava flows of the Chaîne des Puys

AN earlier attempt¹ to use thermoluminescence (TL) for determining the age of Quaternary lava flows, and in particular of the magnetically reversed² Laschamp and Olby flows of the Chaîne des Puys in the French Massif Central, was frustrated by anomalous fading of the TL. This anomalous fading seems to be exhibited by most of the TL-sensitive minerals in lava and is probably due to wave mechanical leakage of electrons out of traps to nearby defects^{3,4}. It has not been observed in quartz¹ but only minimal quantities of this mineral are present in the basaltic flows of the Chaîne des Puys. Although the TL phototransfer technique is a promising⁵ way of using traps that are immune to anomalous fading, it has not yet proved possible to obtain TL dates directly from volcanic lava (although an empirical technique has achieved relative dating in one particular application⁶). We report here the TL dates for the baking by lava of clay and sediment, thereby avoiding the malign TL characteristics of the lava itself. TL dating of baked clay in the form of archaeological pottery is well established⁷ and the procedures developed in that application were followed here. Some of the dates obtained are of particular interest because of the likely association² of one of the lava flows concerned, that at Royat, with the magnetically reversed flows mentioned above. An average TL age for the latter of 33,000 ± 4,000 yr has been obtained by Valladas *et al.*^{8,9}; this is based on quartz extracted from a granitic inclusion found in the Laschamp flow and an underlying quartz pebble heated by the Olby flow.

Table 1 lists the results obtained from two exposures of baked sediment that had been heated by the Royat flow; this flow originated in the region of the Petit Puy de Dôme and moved down the valley of La Tiretaine through Fontanas and Royat to Chamalières (see ref. 10). The upper of these two exposures (samples *j1*, *j3.1*, *j3.2*, *j11* and *j42*—pointed out to M.J.A. by A. Roche in September 1976) lies in Royat on the North side of the Avenue de la Vallée facing the entrance to the car park (on the west side of which is La Grotte des Laveuses). The sediment at this location was plentiful and consisted of well consolidated red baked clay firmly adhering to lava at one boundary. Palaeomagnetic measurements by Barbetti and Flude (personal communication) on two orientated samples of the baked clay collected at the same time as those for TL gave directions within

Fig. 1 Plateau test; the ratio of natural TL to natural plus artificial TL as a function of glow-curve temperature, for *a*, reddened layer (*j1*); and *b*, unreddened layer (above *j1*). Other unreddened layers show similar behaviour. The artificial dose was 9 krad for *a* and 17 krad for *b*.

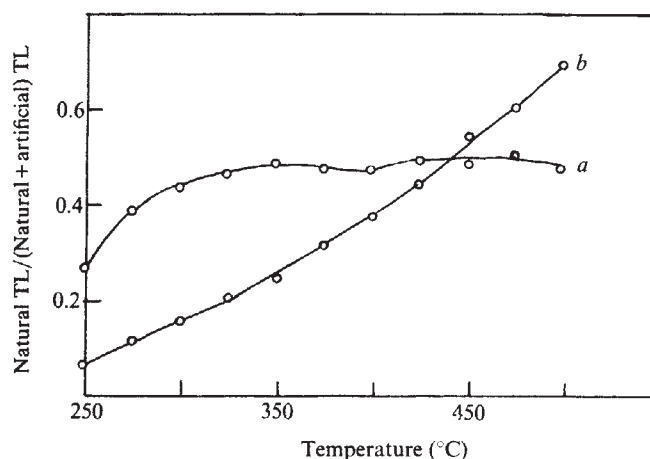


Table 1 Royat locations (45°45' N, 3°03' E)

Sample	Ancient dose (krad)	Water content (% of dry weight)	Dose-rates (rad yr ⁻¹)			TL age (yr × 10 ⁻³)
			Effective α	β	γ + cosmic	
Upper location						
j1	9.0	24	0.125	0.139	0.11	24 ± 2.7
j3.1	11.2	20	0.085	0.132	0.11	34 ± 3.6
j3.2	9.9	20	0.137	0.132	0.11	26 ± 2.7
j11	8.9	24	0.156	0.157	0.11	21 ± 1.8
j42	11.2	26	0.109	0.126	0.11	32 ± 3.2
Lower location						
j32	14.3	27	0.198	0.182	0.12	29 ± 2.8
j34	14.0	23	0.161	0.186	0.12	30 ± 2.6

Mean age (weighted) = 25.8×10^3 yr; standard error = 1.7×10^3 yr; predicted error = 2.2×10^3 yr (random plus systematic).

3° of the rather abnormal result found^{11,12} for lava samples collected from the same location (declination 62° W, inclination +70°, $\alpha_{95} = 3.5^\circ$; location 24 in ref. 12). Similar ancient directions for this flow both in respect of lava and in respect of associated baked sediment were found¹³ some 75 yr ago by Brunhes and David!

The lower location (samples j32 and j34) was on the east boundary of the car park itself, ~5 m below the upper location and 75 m nearer to the centre of Royat (see ref. 14). The sediment here is dark red and only ~1 cm thick; it is overlain by ~30 cm of ashy volcanic debris on top of which is the lava flow itself. The baked sediment here was poorly consolidated and although palaeomagnetic measurements by Barbetti and Flude (personal communication) showed some scatter, the sample mean directions were within 15° of the result previously obtained for the lava^{11,12} (declination 74° W and inclination +63°, $\alpha_{95} = 3.5^\circ$; location CF 22 in ref. 12). A radiocarbon determination by Delibrias *et al.*¹⁵ for carbon extracted from the underlying soil at this locality gave an age of $11,070 \pm 200$ radiocarbon yr (Gif 2255) and on the basis that it is from a well protected palaeosol, this has been taken¹⁴ to be the maximum age for the lava. The palaeomagnetic results from the two locations indicate that they are stratigraphically equivalent.

The TL ages determined for these two locations, given in Table 1, were based on the fine-grain technique¹⁶; the error limits were evaluated as discussed elsewhere^{17,18}. The values for the combined γ + cosmic radiation dose-rates were obtained¹⁹ by burying small capsules of natural fluorite, for a year, in the sediment in a similar position with respect to the lava as had been occupied by the TL samples. Using our standard form of citation the average age derived from all seven samples is 25.8×10^3 yr ($\pm 1.7 \times 10^3$, $\pm 2.2 \times 10^3$, OxTL 132j). The two error limits quoted are^{17,18}, respectively, the standard error of the mean derived from the observed scatter of the individual ages, and the error predicted by taking into account random and systematic sources of uncertainty; the second limit is appropriate when comparing ages derived from other chronometric techniques.

There is a gross discordance between the TL age and the radiocarbon age mentioned above. This could arise in the radiocarbon determination if somewhat more than 20% of

the carbon in the sample was 'modern'. Although this is considered unlikely because of the well protected situation of the palaeosol and because of the pretreatment that was given to the sample to remove contaminating humic acid, this ¹⁴C age should be accepted with caution (G. Delibrias, personal communication, pointed out the desirability in these circumstances of evaluating the age of the humic acids). On the other hand, the TL age might be erroneously too old if there had been infiltration into the sample of unbaked sediment. This is inherently unlikely in the lower location because the overlying lava would have heated any material above the layer of burnt sediment, but error due to this factor is also considered to have been excluded by the following sample rejection procedure.

The presence of a significant amount of unheated material would be expected to cause a sample to fail the 'plateau test' that is routinely applied⁷ (see Fig. 1); this test demands that there is a substantial temperature region (350–450 °C in the present work) of the glow curve within which the ratio of ancient TL to artificially induced TL does not vary (by more than 10% in the present work). Measurements made, for each location, on the ashy material lying above the baked sediment showed a change in this ratio by a factor of about two between 350 and 450 °C; hence the 10% plateau criterion implies rejection of samples in which contamination exceeded 10%. This level might be serious if the equivalent dose carried by the contaminating material was vastly in excess of that carried by the baked sediment. However, the highest equivalent doses evaluated for overlying material were 7 krad at a glow-curve temperature of 350 °C and 18 krad at 450 °C. This corresponds to the overlying material having been heated at the time of the volcanic activity, as might be expected; that the equivalent dose at 450 °C is higher than that for the baked sediment correlates with a higher radioactivity, as observed, and the lower equivalent dose at 350 °C is consonant with the effects of the anomalous fading which, as mentioned previously, prevents TL dating of volcanic material itself. Similar plateau measurements were made on unreddened soil beneath the dated layer at the lower location; these indicated an equivalent dose of 16 krad at 350 °C and 40 krad at 450 °C.

The increase with depth of the equivalent dose (when evaluated at a glow-curve temperature of 350 °C) raises the question of whether the sediment was heated to a sufficiently high

Table 2 St Saturnin (45°36' N, 3°06' E)

Sample	Ancient dose (krad)	Water content (% of dry weight)	Dose-rates (rad yr ⁻¹)			TL age (yr × 10 ⁻³)
			Effective α	β	γ + cosmic	
i12 (quartz inclusions)	3.4	12	0	0.200	0.135	10.1 ± 1.3
i15 (quartz inclusions)	3.0	19	0	0.200	0.135	8.9 ± 1.8
i17 (fine grains)	3.1	24	0.156	0.185	0.137	6.5 ± 0.7

Mean age (weighted) = 8.1×10^3 yr; standard error = 1.1×10^3 yr; predicted error = 0.8×10^3 yr (random plus systematic).

temperature for earlier TL to have been erased. Partial erasure is ruled out by the satisfactory plateau observed for the reddened layers—for partial erasure the degree of erasure at different glow-curve temperatures would vary strongly. Negligible erasure would have been highly unlikely so close to molten lava and can certainly be ruled out for sample *j1* which was actually adhering to a vertical face of lava which terminated the extent of the baked clay.

Accepting that the heating by the lava was sufficient for complete erasure, there is also the possibility that because of anomalous fading the age of 26×10^3 yr may be erroneously too young. Tests on artificially irradiated portions of all the samples listed in Table 1 showed that for a storage period of 6 months any decay was less than experimental uncertainty (3%). However, this does not rule out a significant effect over thousands of years and an attempt was made to utilize the quartz inclusion technique—as there was no evidence that quartz suffers from this malign phenomenon. Unfortunately, the yield of quartz was too small to allow the necessary comprehensive measurements for a reliable date but for sample *j1* the equivalent dose was estimated as 6–9 krad; this corresponds to an age of between 26×10^3 and 35×10^3 yr. Thus although one cannot rule out a small amount of anomalous fading in the fine grains it does not seem to have had a gross effect.

In conclusion, bearing in mind the suggestion² that because of its abnormal magnetic direction, the Royat flow may be associated with the magnetically reversed Laschamp and Olby flows, the TL results for the Royat flow are mutually reinforcing with those obtained by Valladas *et al.*^{8,9} for Laschamp and Olby. Note that the radiocarbon age for the first phase of the geomagnetic polarity excursion²⁰ detected in aboriginal hearths at Lake Mungo, Australia, is $\sim 30 \times 10^3$ yr (5,730-yr half-life), and that the thermoluminescence age²¹ for these hearths is $(33.5 \pm 4.3) \times 10^3$ yr.

We have also carried out TL measurements on clay baked by the lava flow at St Saturnin. The radiocarbon age for this flow, being based on wood carbonised by the lava, is much more secure than the palaeosol determination for the Royat flow. Hence it is of particular interest to ask whether in this case the TL dating is consistent with the radiocarbon determinations. The TL samples, of black baked clay, were collected from above the pathway below the chateau, close to the spot where carbonised wood for ^{14}C dating was obtained by A. Rudel. Unfortunately, the TL characteristics were not all satisfactory and in view of this, the agreement (see Table 2) with the ^{14}C age of $7,650 \pm 350$ radiocarbon yr (Sa90) obtained by Pelletier *et al.*²² is better than could be expected; using the 5,730-yr half-life, the ^{14}C age becomes 7,880 yr and eventual availability of bristle cone pine calibration may show the corresponding calendar age to be greater still. The relevant γ dose-rates were obtained, as for the Royat locations, by burial of small capsules of natural fluorite. Table 2 shows that the standard error is comparable with the predicted error; this reflects the unsatisfactory TL characteristics mentioned above and indicates incidentally that in this application the system for evaluation of predicted error has been insufficiently pessimistic.

Finally, for some samples (provided by G. Camus), measurements were undertaken to check whether or not the flows concerned belonged to the period of Quaternary volcanism. As capsule measurement was not feasible for these samples the γ dose-rate had to be estimated from radioactive analysis of the material presumed to surround the sample. The fine-grain age evaluated for a sample of grès (from the quarry behind 120 Ave du Mont Dore, Beaumont) that had been heated by the Coulée de Boissejour was $\sim 50 \times 10^3$ yr and measurements made on quartz inclusions from this sample were consistent with this. Various other materials from this quarry were tried (baked clay, grès felspathic) but none had sufficiently satisfactory TL characteristics for a reliable TL age to be determined. Clay from Montpoly that had been baked by the Coulée de Beaumont gave a fine-grain age of 34×10^3 yr. For calcaires trempés from the Butte de Clermont the plateau test⁷

indicated that there had been strong fading in the 330 °C peak—indicating either that the material had not been heated in the eruption or that, on the basis of the equivalent dose indicated by the 420 °C peak, it had been heated more than half a million years ago.

We thank Professor A. Roche and Dr G. Camus for help in locating the flows concerned, Dr G. Delibrias for discussion of the radiocarbon dating of the Royat palaeosol, and Dr M. F. Barbetti for his palaeomagnetic results.

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Received 9 May; accepted 28 July 1978.

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Changing He/Ar and N₂/Ar ratios of fault air may be earthquake precursors

THE geochemical method for the prediction of earthquakes depends on the ability to find the anomalous change of fluid phases of the ground, which are expelled by the stress at depth before earthquakes. Here the utility of ratios of He/Ar and N₂/Ar in fault airs is examined in this context.

The radon emanation of groundwater may be a useful indication of the precursory effects of earthquakes¹. Helium may also be of interest, because unlike radon, helium does not decay but accumulates in the lithosphere. Use of only a single element for predicting earthquakes, however, is less reliable in some cases. A rare gas in the groundwater is lost through bubbling² and the concentration of the rare gas is lowered by other gases such as methane and carbon dioxide formed in the ground. Hence, it seems most appropriate to study the relative concentrations among gases and the ratios of He/Ar and N₂/Ar discussed here may prove to be the technically most useful parameters.

Helium and argon produced in the lithosphere are estimated from the abundances of radioactive elements (Table 1), and the ratio of helium to argon found to remain constant for the past several hundred million years irrespective of rock types. The ratio in the lithospheric air (≈ 10) is 10^5 times higher than that in the atmospheric air (5.7×10^{-4}). The ratio in the atmospheric air becomes double the usual ratio because of the contamination of

Table 1 Abundances of U, Th and K in rocks and He/Ar ratios in the lithospheric air derived from the radioactive elements

	U (p.p.m.)	Th (p.p.m.)	K (%)	He/Ar* Myr 100 Myr	
Basalt	0.83†	5†	0.58‡	10.8	10.8
Granite	3.96†	13.5†	2.50§	8.82	8.82
Shale	3.7	12	2.24¶	8.95	8.95

* The year represents the accumulation interval. The decay constants used for the calculation are as follows: $\lambda_U = 1.537 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_{Th} = 4.99 \times 10^{-11} \text{ yr}^{-1}$, $\lambda_K = \lambda_{EC} + \lambda_{\beta} = 5.29 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_{EC} = 5.7 \times 10^{-11} \text{ yr}^{-1}$. The abundance of ^{40}K in potassium is assumed to be 0.0119%.

† General average⁹.

‡ Average in Quaternary Japanese basalts¹⁰.

§ Average in Japanese granite¹¹.

|| General average¹².

¶ Average in Japanese shales¹³.

0.005 vol % of the lithospheric air. Table 2 shows some examples of natural gas composition in Japan and USA. The concentrations of helium and argon are often lower than those in the atmospheric air, whereas the He/Ar ratios are much higher. This indicates that the lithospheric air permeates through the natural gases. The lithospheric air released by the formation of cracks in rocks, therefore, can be easily detected using this ratio.

The ratio N_2/Ar is also expected to be a useful parameter of lithospheric air, because the ratio in natural gases and volcanic gases is generally higher than that of the atmospheric air (85.21) (refs 3–6). The origin of nitrogen in the lithospheric air has been explained in terms of (1) the release of nitrogen by the bacterial decomposition of nitrogen-bearing compounds, (2) the release of nitrogen by the inorganic chemical breakdown of organic materials, and (3) the release of inorganic nitrogen from igneous (and metamorphic) rocks^{4,5}.

The ratios He/Ar and N_2/Ar are measured by gas chromatography without vacuum processes, temperature and pressure corrections and other complicated treatments. This technique is simple enough for amateur observers to make the appropriate measurements.

Since 1976 I have continuously measured the variations of He/Ar and N_2/Ar ratios of gas bubbles found in the water issuing from a fault zone almost 2 m wide which runs in chert and shale of the Palaeozoic. The fault crosses the precinct of Inuyama Crustal Movement Observatory of Nagoya University which is ~20 km north of Nagoya.

The gas bubbles are constantly gathered in a bottle set upside down in the water, then transferred into a glass flask with high vacuum stopcocks. Some of the gas sample (2 ml) is injected into the gas chromatograph, tank oxygen is used as a carrier gas. Helium, argon and nitrogen are separated through a molecular sieve 5 Å column at a room temperature, and the three peaks of these gases emerge on a recorder chart within 10 min. The relative areas of peaks correspond to the relative volumes of

gases in the sample and the mutual ratios among these gases are determined by the atmospheric air as a reference standard in the same conditions. Error (2σ) in the determination is ~10% for He/Ar ratios and ~2% for N_2/Ar ratios. If the gases were each measured by a different method, the error would inevitably be increased. The single procedure provides sufficient precision for analysing the ratios.

The successive measurement indicated that a high He/Ar ratio (0.014) was accompanied by the earthquake of 26 July 1976 (magnitude: 4.1; epicentral distance: 67 km), but the sampling interval (once or twice a month) was too long to decide whether the high ratio occurred before or after the shock⁷. The sampling interval was therefore shortened after March 1977 (once in two or three days).

Some correlation between occurring of earthquake and change of gas quality was observed, but the variation of the observed ratios has no relationship to the records of tiltmeter, extensometer and precipitation obtained in the observatory. The ratios of He/Ar and N_2/Ar fluctuated considerably: for the next 10 measurements, the moving average for data smoothing was calculated after March 1977. The results are plotted in Fig. 1, which shows that a few peaks appeared. The four vertical

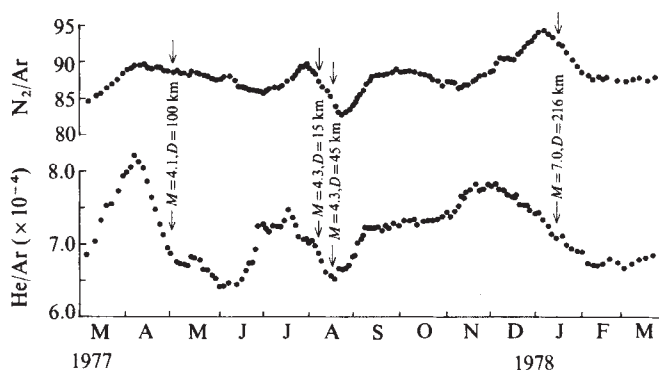


Fig. 1 The temporal variations of He/Ar and N_2/Ar ratios in gas bubbles from the fault zone. The original data sequence was smoothed by 10-term moving averages after the usual process in statistics¹⁴. The vertical arrow indicates the earthquakes with the magnitude >4 in the vicinity of Nagoya. M represents magnitude and D , epicentral distance. See also Table 3.

arrows show the earthquakes (magnitude ≥ 4) which occurred in the vicinity of Nagoya during the observation. The three events of the four were felt in Nagoya (Table 3). Figure 1 shows explicitly that the peaks appear before the earthquakes. This correlation found can hardly be chance and suggests that the change of gas composition is closely related to the occurrence of an earthquake. The epicentral distance of the earthquake on 14 January 1978 amounts to 216 km, which greatly exceeds the size

Table 2 Examples of natural gas compositions

No.	CH ₄	C ₂ H ₆	N ₂	CO ₂	He	Ar	He/Ar	N ₂ /Ar
			(%)					
<i>a</i>								
KR-5-2	97.1	2.11	0.48	0.17	0.00055	0.0060	0.092	80
KR-9-2	96.9	1.87	0.43	0.59	0.00066	0.0039	0.17	110
<i>b</i>								
2	95.43	2.37	1.57	0.09	0.0041	0.0052	0.79	302
3	95.12	3.96	0.86	0.06	0.0037	0.0067	0.55	128

a, Nishinagaoka, Niigata, Japan^{3,4}.

b, Rio Vista, Sacramento, California⁵.

Table 3 The earthquakes (magnitude ≥ 4) occurred in the vicinity of Nagoya during the measurements of Fig. 1

Date	Magnitude	Distance from the epicentre (km)	Intensity in Nagoya*
30 April 1977	4.1	100	0
6 August 1977	4.3	15	III
15 August 1977	4.3	45	I
14 January 1978	7.0	216	III

* Intensity expressed on the scale of the Japanese Meteorological Agency.

of the dilatant volume generally accepted. According to the observation in China⁸, however, anomalous radon contents of groundwater were recorded at distances less than 110 km from the shock, for events in the magnitude range 4.3–5.0; for the larger quake of magnitude 7.9 in the Pohai Gulf, the wells showing radon anomalies were located at distances of 250–300 km. These observations suggest that the changes in gas composition reflect sensitively stresses which precede earthquakes and such changes could be precursors of earthquakes.

Several aspects arise from Fig. 1, in terms of earthquake prediction. The duration of the high ratio is longer for one large earthquake (14 January 1978, magnitude 7.0) than those for the small ones (magnitude ≈ 4). Another aspect is that the peaks of He/Ar precede those of N₂/Ar for all events. This might be attributed to the difference in diffusion velocity of He and N₂ through capillary cracks formed by stress before earthquake. The molecular weight of N₂ is 28 and of He is 4; the ratio of diffusion velocities is $\sqrt{28/4} = 2.65$ and He migrates more rapidly by diffusion than does N₂. It is also noticeable that the time lag of the N₂/Ar peak for the large earthquake is longer than for the others. Thus, by estimating both the time lag of N₂/Ar peak and the duration of high ratios, one could predict the occurrence time and magnitude of the approaching earthquake. Furthermore, observation in many locations may provide a basis for predicting where the earthquake will occur.

The earthquake of 14 January 1978 caused a disaster in the Izu Peninsula. Many observatories and seismology and geodesy instruments such as tiltmeter, extensometer and seismograph have been installed around the peninsula. These instruments did not record the premonitory phenomena for the earthquake except for a radon meter and a Sacks–Evertson strain meter. On the other hand, the method described here clearly records the precursory changes in gas compositions at the epicentral distance of 216 km. It seems therefore that this method is a promising practical means of predicting earthquakes.

I thank Drs R. Shichi and Y. Fakuo for assistance and helpful suggestions.

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Received 10 May; accepted 24 July 1978.

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Seawater/basalt ratio effects on the chemistry and mineralogy of spilites from the ocean floor

PILLOW basalts metamorphosed to the greenschist facies have been frequently dredged from mid-ocean ridges^{1–3}. These rocks usually exhibit a distinct mineralogical differentiation in which the pillow rim or margin is replaced by chlorite or, less commonly, epidote. Calcic plagioclase and olivine within the pillow interior or core are replaced to varying degrees by albite and chlorite, respectively². Actinolitic amphibole, pyrite, quartz, sphene, and small amounts of nontronite and saponite are the other alteration phases identified in these rocks^{1–3}. Corresponding to this mineralogical differentiation is a chemical differentiation characterised, in general, by enrichment of MgO and H₂O in the pillow rim relative to the core; the core is in turn enriched in Na₂O, CaO and SiO₂. Cann², in his study of the metamorphosed pillow basalts dredged from the Carlsberg Ridge, Indian Ocean, refers to the Mg-enriched, Ca-depleted, chloritised rind of the pillows as hyalospilites, and the Na-enriched cores as orthospilites. Metabasalts similar in chemistry and mineralogy to those dredged from mid-ocean ridges have also been found in ophiolites, obducted portions of oceanic crust now exposed on continents^{4,5}. The oxygen- and strontium-isotopic composition of these basalts^{6,7}, and the oxygen-isotopic composition of secondary minerals separated from dredged basalts⁸, suggest that alteration resulted from interaction with seawater at temperatures from about 200 to 350 °C. Passage of seawater through a sequence of pillow basalts would most probably occur along fractures and cracks between individual pillow tubes. In this regard, Spooner *et al.*^{6,7} from studies of the extent of hydration, oxidation and oxygen- and strontium-isotopic composition of metamorphosed pillow basalts at Troodos, Cyprus, have concluded that the flow of seawater through basalt pillow piles occurred primarily along fractures and pillow boundaries, whereas grain-boundary diffusion was the principal mechanism of mass transfer accompanying chemical reaction within the solid rock. Because of this variation in permeability, pillow rims would be exposed to a much greater volume of seawater relative to the crystalline cores; consequently, alteration of the rim would proceed at a higher effective water/rock ratio⁹. We propose here that the water/rock mass ratio during seawater–basalt interaction affects the stability of secondary minerals and therefore accounts for

Table 1 Chemical composition of basalt glasses

Component	Fresh	Altered*	Altered†
SiO ₂	50.10	46.44	43.36
Al ₂ O ₃	14.80	14.18	13.74
Fe ₂ O _{3(t)}	12.60	12.30	11.50
MgO	7.00	8.59	15.24
CaO	11.10	7.84	1.64
Na ₂ O	2.57	2.59	0.89
K ₂ O	0.14	0.04	0.06
TiO ₂	1.78	1.69	—
H ₂ O ⁺	0.50	6.40	11.00
Total	100.59	100.07	97.43

—, Not analysed.

* Altered basalt glass after interaction with seawater at 350 °C, water/rock ratio 10.

† Altered basalt glass after interaction with seawater at 300 °C, water/rock ratio 62.

the mineralogical and chemical diversity between the core and rim of metamorphosed pillow basalts. To demonstrate this, we report the change in mineralogy and chemistry of basalt glass reacted with seawater at 300 and 350 °C, 500 bar at water/rock mass ratios of 62 and 10, respectively. The experimental system and analytical procedures are discussed in refs 10, 11.

Interaction of seawater with basalt glass at 350 °C, 500 bar, and water/rock mass ratio of 10 results in the formation of a mineral assemblage containing albite, saponite, mixed-layered chlorite-saponite, actinolitic amphibole and anhydrite, as well as minor prehnite, quartz and pyrite. In general, this alteration assemblage is similar to that in the cores of metamorphosed basalts. Furthermore, because of chemical exchange with seawater during this experiment, the basalt glass gained Na₂O, MgO and H₂O and lost CaO, SiO₂ and K₂O, while total iron and Al₂O₃ were conserved (Table 1). These directions of chemical

Table 2 Gains (+) and losses (–) of chemical components resulting from formation of orthospilite or hyalospilite from fresh Indian Ocean Ridge basalt², and during experimental spilitisation of basalt glass

Component	Orthospilite	Basalt glass*	Hyalospilite	Basalt glass†
SiO ₂	+7.02	–1.63	–7.77	–3.39
Al ₂ O ₃	—	—	—	—
Fe ₂ O _{3(t)}	+0.19	–0.10	+10.78	+0.46
MgO	+1.15	+1.97	+4.98	+9.46
CaO	–2.16	–2.92	–8.93	–9.32
Na ₂ O	+1.88	+0.13	–1.14	–1.61
K ₂ O	–0.06	–0.10	–0.08	–0.08
H ₂ O ⁺	+2.83	+6.18	+7.76	+11.38

Al₂O₃ has been conserved.

* Spilitisation at seawater/basalt mass ratio 10.

† Spilitisation at seawater/basalt mass ratio 62.

exchange, and for some species even the magnitude, are strikingly similar to those inferred during the transformation of tholeiitic basalt to orthospilite (compare columns 1 and 2 of Table 2). The one exception is SiO₂, which is enriched in the orthospilite samples described by Cann². During experimental 'spilitisation', SiO₂ is lost from basalt because fresh seawater contains very little SiO₂ and the tendency is for the solution to become saturated with quartz. Whether a rock in the natural setting becomes enriched or depleted in SiO₂ depends in general on whether the solutions which pass through it are super-saturated, as in the case of a cooling solution, or undersaturated with quartz. The enrichment observed by Cann may thus not be typical for orthospilites as a whole.

Further evaluation of the data (Table 2) reveals that, although Na is enriched in basalt during this experiment, the degree of enrichment is minor compared with that of the typical orthospilite. The formation of albite from basalt glass or calcic plagioclase depends critically on the Na/Ca ratio of the aqueous phase. If this ratio is large and remains large during seawater-basalt interaction, the basalt would generate potentially greater amounts of a sodic phase such as albite at the expense of calcic phases such as epidote or prehnite. Although seawater has an initially high Na/Ca ratio, reaction with basalt within a closed system results in a significant decrease in this ratio and in the tendency to form albite. For example, in the present experiment the Na/Ca molal ratio of the solution decreased from 45 to 13, partly by loss of Na but mainly by gain of Ca (M.J.M. and W.E.S., unpublished data). In nature, however, this build-up of Ca in the intergranular 'pore' solution within the crystalline core would be free to diffuse into zones having a lower Ca concentration and a higher Na/Ca ratio, such as the relatively less altered, more freely circulating seawater infiltrating interpillow channels (Fig. 1). Concomitantly, Na in this fluid could then

diffuse into the core and become fixed as albite; this process would account for the Na-rich chemistry of orthospilites. It is important to note, however, that if circulation of seawater within the interpillow channels is impeded for some reason, a decrease in Na/Ca ratio can be expected. This would alter the diffusion gradients of Ca and Na and ultimately result in the formation of calcic silicates as vein fillings. Phenomena of this sort may help to explain the 'epidote-rich' greenstones reported by Humphris and Thompson³, in which epidote typically occurs in veins.

Seawater-basalt interaction at 300 °C, 500 bar, and a water/rock ratio of 62 represents a seawater-dominated system¹², in which most of the CaO, Na₂O and K₂O as well as some of the SiO₂ are leached from the basalt (Table 1). At the same time, MgO and H₂O are added to the altered basalt, which was converted entirely to a mixed layered chlorite-smectite. The only other phases which formed were anhydrite and very minor haematite.

The exchange of components between seawater and basalt during alteration at a high water/rock ratio agrees well with the chemical gains and losses inferred from the chemistry of the chloritised rind (hyalospilite) of pillow basalts dredged from the Carlsberg Ridge (Table 2). Furthermore, interaction at a high water/rock ratio produces a solution appreciably enriched in Fe, Mn, Cu, Zn and SiO₂. The precipitation of these components, either in channels within the pillow pile or on the seafloor could explain the widespread jasper and ferromanganous sediment commonly associated with spilitised basalt.

The mineralogical and chemical heterogeneity commonly observed in metamorphosed seafloor basalts can, therefore, be explained on the basis of interaction with seawater at various water/rock ratios. Experimental seawater-basalt interaction at

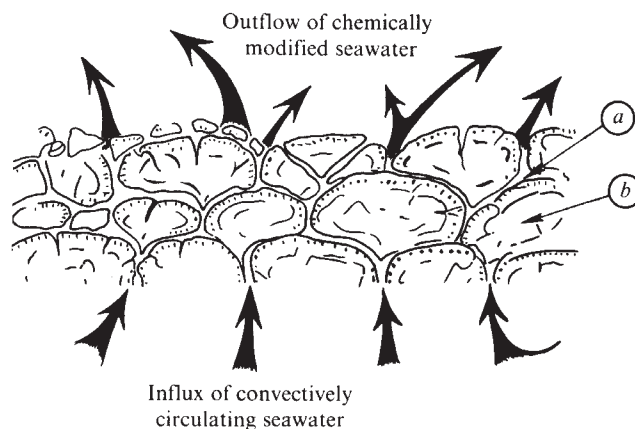


Fig. 1 Schematic illustration depicting circulation of seawater through pillow basalt pile undergoing hydrothermal alteration: *a*, primary zone of Mg-metasomatism (high water/rock ratio); *b*, zone of alteration characterised by diffusional metasomatism (low water/rock ratio).

a low effective water/rock ratio produces an alteration assemblage characterised by albite, actinolite, prehnite, chlorite-smectite, quartz, pyrite and anhydrite. At a high water/rock ratio (62), in contrast, the basalt is strongly leached and replaced by chlorite-smectite. These results apply directly to the natural system, in which, because of the inherent permeability of pillow basalt piles, the pillow core has limited direct access to seawater and alters at a low water/rock ratio, whereas the glassy selvage of the pillow exterior apparently comes into contact with much greater volumes of seawater and consequently alters at a high water/rock ratio.

We thank Robert G. Coleman and Dave Piper of the US Geological Survey for critically reviewing the manuscript. This research was performed in the Stanford University Hydro-

thermal Laboratory, and at the US Geological Survey, Menlo Park, California. Funding was provided by NSF grant ID 074-12880.

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Received 23 June; accepted 13 July 1978.

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Adaptive value, entropy and survivorship curves

NATURAL survivorship curves fall into three main types^{1,2}. Type I, or rectangular distribution, describes the situation in which all individuals attain the maximum physiological longevity of the species. Here the maximum age at death and the mean life expectancy coincide. The Type II life table describes a mortality which is independent of age; that is, no single age group is favoured at the time of dying. The Type III table is characterised by a high mortality early in life and a life expectancy which increases with the age of survivors. The three types of curve are illustrated in Fig. 1. The general shape of the survivorship curve is fixed for each species, however, the convexity of the curve is highly sensitive to environmental conditions and the genetic constitution of the population. This fact suggests that the general shape of the life table may provide information about the genetic variability in the population, the range of environmental factors that impinge on the population or the incidence of random events in the lifetimes of different individuals³. This note characterises in terms of two new demographic parameters the relative effect genetic and environmental factors play in the evolution of the life table. The analysis we give revolves around two demographic variables, the entropy of a population and the adaptive value. Entropy H measures the variability of the mortality distribution. The measure of variability also describes the convexity of the life table. The adaptive value ψ is a measure of the correlation between the variability of the mortality distribution and the environmental variability. Thus the adaptive value reduces to the entropy when the environment is constant.

We give two distinct characterisations of life-history curves in terms of optimisation arguments. In the first characterisation, we assume that the survivorship curves are completely determined by the genetic constitution. The life tables are derived by maximising H subject to constraints which are given a genetic interpretation. In the second description, we assume that the environmental effects are the only significant influence. The three life tables are derived in terms of maximising ψ subject to constraints on the environmental parameters. We

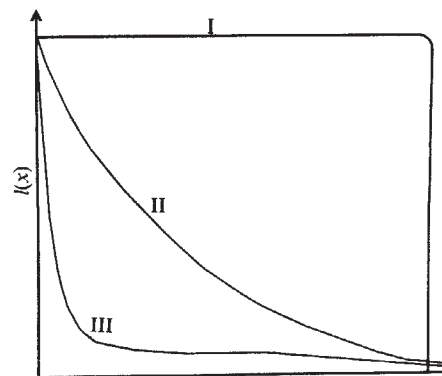


Fig. 1 The theoretically possible distinct types of life table. For Type I, $H = \log e^0$; for Type II, $H = 1 + \log e^0$ and $l(x) = \exp -(x/e^0)$; for Type III, $H = \frac{1}{2} + \log e^0$ and $l(x) = \exp -(\pi/4e^{02})x^2$.

also discuss the corresponding biological mechanisms which generate the life tables. Note that the genetic and environmental constraints which are imposed in the optimisation arguments correspond to initial conditions in the mechanistic description. These biological mechanisms can be mathematically described in terms of a birth and death process. This mathematical model together with a precise description of the correspondence between constraints and initial conditions is discussed elsewhere⁶.

Let $l(x)$ be the probability that an individual born at age zero survives to age x , and let $m(x)$ be the age-specific fecundity. The net reproductive rate R_0 is given by

$$R_0 = \int_0^\infty l(x)m(x) dx$$

The expectation of life of an individual aged x is

$$e^0 = \int_0^\infty l(x) dx$$

The entropy of the population⁴ is given by

$$H = - \int_0^\infty q(x) \log q(x) dx$$

where $q(x) = l(x)m(x)/R_0$ is the probability density function of the age of reproducing individuals. The expression H is a measure of the variability of the contribution of the different age-classes to the stable age-distribution. It also measures the convexity of the net-maternity function $V(x) = l(x)m(x)$. Fixing the fecundity, say $m(x) = 1$, we obtain

$$H = - \int_0^\infty \frac{l(x)}{e^0} \log \frac{l(x)}{e^0} dx \\ = - \frac{\int_0^\infty l(x) \log l(x) dx}{\int_0^\infty l(x) dx} + \log e^0$$

Normalising, we obtain

$$H^* = - \frac{\int_0^\infty l(x) \log l(x) dx}{\int_0^\infty l(x) dx}$$

The expressions H and H^* measure the convexity of the survivorship curve. For the Type I life table, $H^* = 0$ and $H = \log e^0$. For the Type II life table, $H^* = 1$, and $H = 1 + \log e^0$.

An interesting application of our entropy parameter to the study of male-female mortality patterns is given in ref. 5.

The notion of an adaptive value of a population⁶ generalises the entropy concept to incorporate the effects of environmental forces on the life-history distribution. We refer to ref. 6 for a precise mathematical definition. In this note we give an intuitive account. We consider the population as a stationary stochastic process whose phase space, the set of all life histories is subject to perturbations by an environmental process. There

are thus two stochastic processes at work, the process described by the population and the process described by the distorting effects of the environmental action. We can therefore identify two entropies, H , the entropy of the population in the case when no environmental forces interfere, and $\hat{H}$, the conditional entropy, that is, the entropy of the perturbed process given that the unperturbed mortality distribution is known. The adaptive value ψ is defined to be

$$\psi = H + \hat{H}.$$

This function measures the correlation between the variability of the mortality distribution and environmental variability. Thus, in a constant environment, ψ is precisely the entropy H of the population.

We now describe the various distributions that maximise H subject to various constraints. We suggest biological interpretations of these constraints by specifying mechanisms which implement these adaptive strategies.

(1) The distribution that maximises H subject to the constraint

$$\int_0^{\omega} l(x) dx = 1$$

where ω is the maximum life expectancy, yields the Type I life table.

The mechanisms implementing this strategy can be described in terms of a mortality which is the end result of senescence or wearing out of the individual, the senescent process going on at the same time and at the same rate in each individual.

(2) The distribution that maximises H subject to the constraint on the mean of the distribution $l(x)$ is given by

$$l(x) = \exp\left(\frac{-x}{e^0}\right)$$

This yields the Type II life table. This curve describes great interindividual variation in the age at death. The variation can arise exclusively from interindividual genetic variability. In the Type I curve, there is no genetic variability, hence the senescent process is the same in each individual. This implies that the life expectancy and the maximum longevity of the species coincide. For the Type II curve, there is genetic variability, hence senescence is less uniform. The degree of genetic variability of the population is measured by the constraint on the mean of the mortality distribution.

(3) The distribution that maximises H subject to a constraint on the variance is given by

$$l(x) = \exp\left[\frac{-\pi}{4e^0}x^2\right]$$

Of the three curves described, Type III represents the greatest individual variability in the time of dying. The constraint on the variance is a measure of the genetic variability in the population. The senescent process in this case is much less uniform than that which generates the Type II life table.

It is important to note that these distributions can also be derived as a result of maximising the adaptive value ψ , subject to environmental constraints. Thus an environmental agent that is lethal to individuals irrespective of their genetic constitution and that kills off all individuals at some prescribed age ω will yield a life table whose variability is maximally correlated with the environmental action, that is, a Type I curve.

An environmental agent that acts randomly on the individuals of different ages and is indifferent to the ages of the individuals will yield a Type II curve. Finally, an environmental agent that is age-specific and selectively removes younger individuals will yield a Type III table.

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Received 5 June; accepted 30 June 1978.

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Egocentric orientation is influenced by trained voluntary cyclorotary eye movements

A CYCLOROTARY eye movement is a motor response of the eye made around the visual axis. Counter-rolling of the eye, for example, occurs during lateral head tilt^{1–5}; conjugate rotary nystagmus can be induced by a large rotating field^{6–9}; and disjunctive cyclorotations can occur during ordinary convergence^{10,11}. Because none of these torsional eye movements can be produced as an isolated voluntary response, eye torsion has always been classified as an involuntary response, a reflex. Using a visual-feedback procedure, however, we have trained humans to make conjugate voluntary cyclorotational eye movements up to 30 degrees in magnitude¹². We have also demonstrated that these large torsional movements are not visually induced and can be made in the absence of any visual stimulus. Accompanying the training and performance of these eye movements were a number of striking illusions related to one's own sense of body orientation. Because these newly trained eye movements are unprecedented, it is of interest to characterise accompanying illusions in detail, comparing them with other illusions of self rotation induced through vestibular¹³ and visual^{14,15} inputs. In this paper we compare the effects of trained cyclorotary eye movements with head and whole body tilts, showing a quantitatively similar change in egocentric orientation for each type of tilt. As such, our findings suggest the possibility of shared mechanisms affecting the stability of one's internal frame of reference, both for eye and body movements.

The exact method of training and testing of voluntary torsion using visual biofeedback has been detailed elsewhere¹². A subject was seated in a dark room with head movements fixed by a full mouth bite plate. A vertical 11 × 0.25° flash was presented monocularly to generate a vertical afterimage. This afterimage was then imaged in space next to a line of light of equal colour, brightness and size (real line stimulus). The subject was instructed to keep this afterimage line matched parallel to the real line only by cyclorotating the eyes. With practice, the subject gradually acquired the ability to rotate the eye(s) to match greater and greater inclinations of the real line. These cyclorotations were trained at the rate of about 0.8° h⁻¹ d⁻¹ for 35 d (Fig. 1a). Using similar methods for 5–10 h of additional training, subjects were trained to make torsional slow pursuit (Fig. 1b) and torsional saccades (Fig. 1c). Individual frame analysis of 35-mm slides or 16-mm motion pictures filmed simultaneously were used to objectify the cyclorotary eye movements. Measurements from photographs used radial iris markings or limbal-scleral blood vessel junctions in relation to a visible and stable reference marker. Accuracy of measurements was ± 10 min.

During initial training, but not during final experimentation, subjects often felt that their heads and bodies were 'rolling laterally' and sometimes experienced associated visual illusions. Accompanying these changes in egocentric orientation were intermittent sensations of stomach nausea, headache and general body fatigue. The most dramatic of these illusions occurred as a distinct 'hallucinatory barrage' at least twice to each subject during initial training. Associated with eyelid tremor, the nearly vertical and parallel lines would appear to move independently, becoming almost horizontal and also appearing curved or wiggly. Accompanying these peculiar visual illusions were very powerful sensations of body flotation

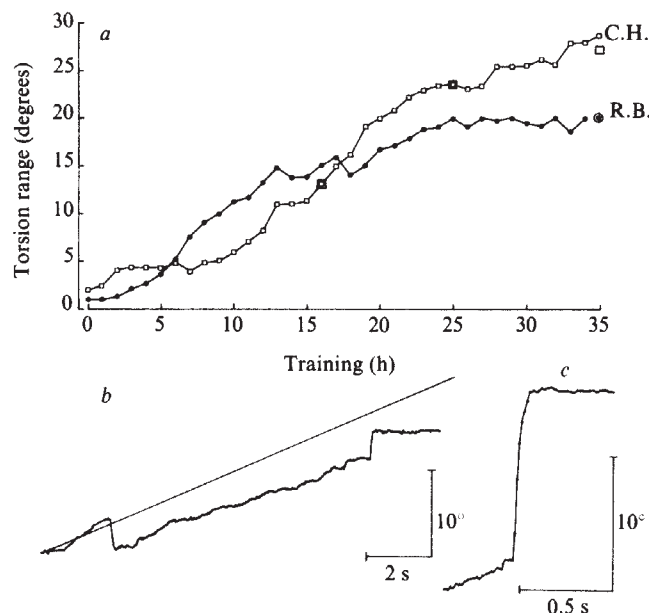


Fig. 1 *a*, Training of voluntary torsion. Maximum torsional range measured psychophysically (small symbols) or photographically (large symbols) plotted against hours of training. *b*, Voluntary cycloversional slow pursuit. Solid line represents a tracing moving at the correct velocity of 1.6° s^{-1} . Photographed at 24 frames s^{-1} . *c*, Time course of a voluntary torsional saccade. Photographed at 64 frames s^{-1} .

and/or rapid downward falling in the direction of the voluntary cyclotorsion. Although self-induced, these sensations show similarities to those experienced by pilots in conditions in which conflicting visual and orientational information can occur¹⁶. It should be noted that these gross and episodic disorientations occurred only during initial training.

In this report, we restrict our quantitative observations to a more constant and milder sensory illusion of body tilt, persisting even after a long period of training. To examine this shift in egocentric orientation and to make comparisons with other situations involving vestibular and neck proprioceptive input, we have devised a control and three test situations. Two subjects felt a rod pivoted about its centre so as to rotate in the frontal plane and adjusted it to the apparent vertical in the dark under each of the following conditions (Fig. 2, top): (1) the real line stimulus was viewed in otherwise dark surroundings at one of several angles of tilt (S); (2) voluntary eye torsion was used to match an otherwise vertical afterimage to specific tilts of the real line (E); (3) a head tilt was used to match the orientation of the real line to that of the afterimage (H); and (4) head and body were both tilted by means of a rotating chair in complete darkness (B).

All tilts and rotations were presented in 3° increments over a range of $\pm 9.0^\circ$. In condition H, the reflexive counter-torsion of the eye was less than 1° (average 0.6°) for even the largest 9° tilts; consequently, actual tilts of the head are slightly underestimated. Before each trial, the experimenter randomly displaced the rotatable rod to ensure that subjects could not memorise hand or rod positions.

The results are graphed in Fig. 2 (bottom). Condition S, as a control, demonstrated that the presence of the inclined real stimulus line had essentially no influence on the subjects' sense of vertical, changing it by only 3% of the real line's tilt. Such a line has also been shown to induce essentially no torsional movements of the eyes¹². Therefore, it can be concluded that the presence of the inclined real line in conditions E and H is of little consequence.

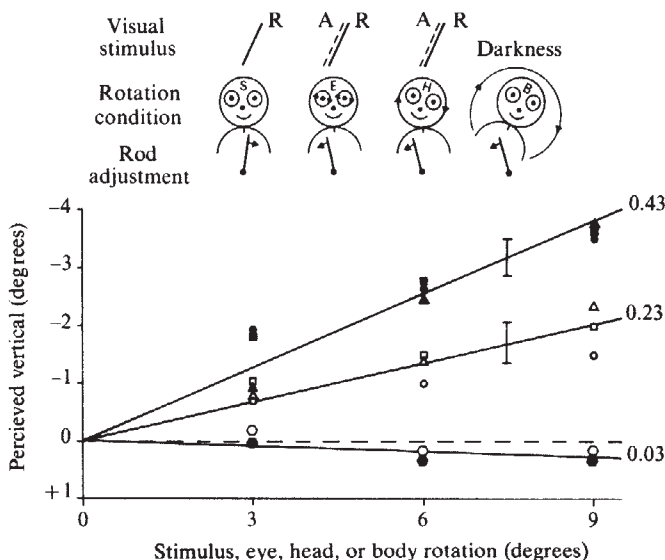
Voluntary torsion (condition E), however, caused marked shifts in the position of apparent vertical as did the two other

conditions (H and B). All adjustments were overestimated in the opposite direction to eye, head or body tilts and by an amount which was virtually identical in all three conditions (Fig. 2). It should also be noted that the results for conditions H and B confirm previous reports^{17,18}. Because the results are so similar in the three conditions, we averaged the data and plotted a single regression line for each subject, arriving at a slope of 0.23 for subject C.H. and 0.43 for subject R.B. If 1.0 is defined as a gain factor whereby a perfect vertical rod adjustment could be made, a gain factor for the subjects' sense of vertical or egocentric orientation can be determined by adding 1.0 to each slope. Thus, C.H. and R.B. overestimated their turning by an average factor of 1.23 and 1.43, respectively.

These experiments suggest that for each subject, there is a constant overcompensation signal determining egocentric orientation, regardless of whether the eye is voluntarily rotated (voluntary cyclofixation), the otoliths and neck proprioceptors are stimulated (head tilt condition), or the otoliths are stimulated without significant neck proprioception stimulation (whole body tilt). In this case of voluntary torsion, as well as for other examples of the perceptual effects of eye torsion^{17,19}, the compensatory signal could arise either as an outflow, originating from a torsional command centre, or as an inflow, originating from eye muscle receptors. The results do not indicate the source of the compensatory signal.

What is surprising is that the motor act of voluntary torsion, which is independent of vestibular or visual stimulation, can, by itself, induce quantitatively identical sensations to actual head and body rotations. This similarity suggests that there might be a common pathway for each effect. Electrophysiological

Fig. 2 A laterally rotatable rod was adjusted to perceived vertical in the dark by subjects in different conditions of rotation over a range of $\pm 9^\circ$ (top). The visual stimulus for each condition is shown: R, real line; A, afterimage line. Both R and A are set to vertical when eyes are at rest and straight ahead. Rotation conditions are defined as follows: S, real line stimulus rotation only (control); E, voluntary eye torsion defined by an afterimage match to tilts of the real line; H, head tilt defined by an afterimage line match to tilts of the real line; B, body and head tilt in complete darkness. Graph shows the change in perceived vertical as a function of rotation. Rod settings for conditions E, H and B were in the opposite direction to any direction of physical rotation (abscissa) by a factor of about one-third (average slopes of 0.23 and 0.43 for subjects C.H. and R.B.). Control condition (S) showed essentially no effect (average slope = 0.03). $n = 40$ for each data point. Error bars on regression lines indicate average s.e. mean for the combined data of C.H. (bottom line) and R.B. (top line), based on 468 df. R.B.: $\bullet$, S; $\blacktriangle$, E; $\blacksquare$, H; $\bullet$, B; C.H.: $\circ$, S; $\triangle$, E; $\square$, H; $\circ$, B.



recordings from the vestibular nerve, for example, show that moving visual scenes produce signals that are similar to a motion stimulus to the semicircular canals²⁰⁻²³, a result which could be the physiological basis for the recent finding that egocentric orientation can be altered by rotating visual fields¹⁴. Perhaps a similar sharing of circuitry exists with respect to the newly trained response of voluntary eye torsion.

This work was partially supported by the NEI (grants EY01582 and EY01186) and by the Smith-Kettlewell Eye Research Foundation.

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Possible role of excretory/secretory products in evasion of host defences by *Fasciola hepatica*

RATS develop a marked resistance to reinfection with *Fasciola hepatica* after a single immunising infection^{1,2}. This resistance is apparent within 2 weeks of initial infection^{3,4} but diminishes in long-standing infections⁴. Resistance does not depend on the presence of the initial infection at the time of challenge², and operates against intraperitoneally implanted metacercariae, juvenile or adult flukes³ as well as against orally administered metacercariae. Implantation (subcutaneous or intraperitoneal) with adult flukes does not stimulate marked resistance to challenge^{3,5}, and these implanted flukes may persist for many months in the cysts which form around them in the rat. Resistance may be transferred by cells⁶ or, provided that it is given at the time of challenge, by immune serum⁷; serum administered 6-8 d after infection is not effective⁷. However, the flukes of the immunising infection persist in the rat in an apparently normal healthy condition^{1,2} and clearly evade the mechanism which controls challenge infections. We report here experiments which show that *F. hepatica* produces substances which are toxic to its host's lymphocytes and that these substances may protect the parasite from its host's immune defences.

Liver fluke excretory/secretory products (ESP) were obtained by incubating adult flukes, collected from sheep or rats, in dry, stoppered plastic tubes for 1-2 h at 37 °C. During this time the flukes expelled the major part of their caecal contents. This was separated by centrifugation and stored at -20 °C until required for use. ESP were tested for toxicity to rat splenic lymphoid cells using the method of van Furth and van Zwet⁸ and

Table 1 Effect of ESP on immunoadhesion of peritoneal cells to *F. hepatica*

Replicate		% Flukes with adherent cells		ESP
		Control	Immune control	
1		0	91.7	11.1
2		0	73.8	16.7
3		14.3	61.5	4.3
4		10	76.0	8.3
5		0	76.2	14.3
Mean		4.9	75.8	10.9

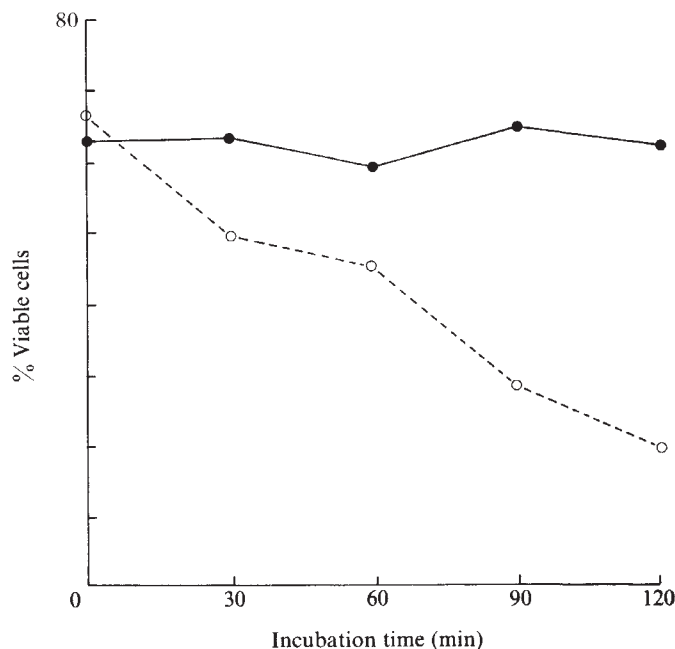
0.1-ml volumes of juvenile *F. hepatica* (~2,000 per ml) and immune rat peritoneal cells (6×10^6 per ml) in tissue culture medium 199 were incubated with 0.1 ml fresh normal rat serum + 0.1 ml medium 199 (control), 0.1 ml fresh immune rat serum + 0.1 ml medium 199 (immune control) or with 0.1 ml fresh immune rat serum + 0.1 ml ESP (ESP). The % of flukes with adherent cells was noted after 90 min incubation at 37 °C. Mean of control compared with mean of immune control, $P < 0.001$; mean of immune control compared with mean of ESP, $P < 0.001$.

the results are shown in Fig. 1. Viability of control cells remained at the fairly constant level of about 60% throughout the 2-h incubation period, whereas cells incubated in the presence of ESP showed a decrease in viability from 66% preincubation to 19% after 2 h.

The relevance of this finding to the evasion of host defences by *F. hepatica* was demonstrated using an *in vitro* immunocytoadhesion system. Juvenile *F. hepatica* were obtained by the *in vitro* excystation of metacercariae. When incubated *in vitro* with fresh serum and peritoneal cells from *F. hepatica*-resistant rats, these juvenile flukes become heavily coated with cells which include many eosinophils (Fig. 2). No cellular adhesion is seen if serum from uninfected rats is used.

The effect of including *F. hepatica* ESP in this *in vitro* system is seen in Table 1. Incubation in normal rat serum gave a mean of 4.9% flukes with cells attached, whereas incubation in immune serum gave 75.8% flukes with attached cells. Inclusion of *F. hepatica* ESP as well as immune serum reduced the numbers of flukes with adherent cells to 10.9%. This reduction was statistically highly significant (Student's *t*-test).

Fig. 1 Toxicity of ESP to lymphoid cells *in vitro*. 0.2-ml volumes of normal rat splenic lymphoid cells (15×10^6 per ml) in tissue culture medium 199 were incubated with 0.2 ml medium 199 (solid line) or 0.2 ml ESP (broken line). At 30-min intervals equal volumes of cell suspension and 0.2% Trypan blue were mixed and the % of viable cells determined.



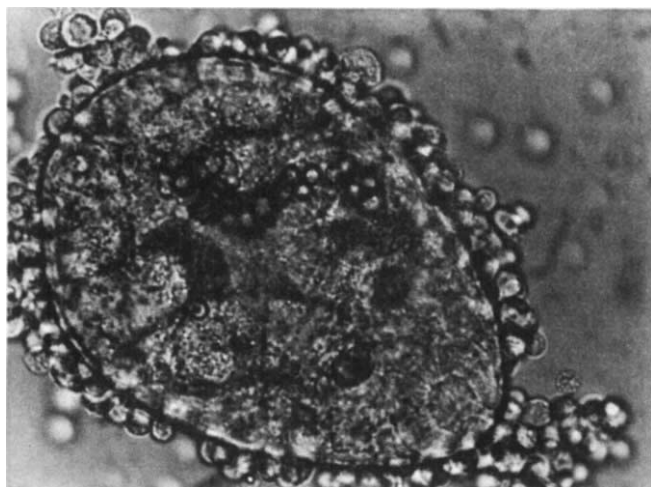


Fig. 2. Immune adhesion of peritoneal cells to *F. hepatica*. 0.1-ml volumes of juvenile *F. hepatica* (~2,000 per ml) and immune rat peritoneal cells (6×10^6 per ml) in tissue culture medium 199 were incubated at 37°C for 15 min with 0.1 ml fresh immune rat serum. Wet preparation 400× magnification.

Cellular attachment is likely to be a first step in the immune killing of *F. hepatica* and the above results clearly show that *F. hepatica* ESP prevent this attachment in the *in vitro* situation. It seems highly probable that ESP also have a similar role *in vivo*, where they are maintained in intimate contact with the fluke in the bile duct or in the fluke's migratory tract in the liver.

It is thus proposed that the actively feeding liver fluke protects itself from host immune defences by producing substances which are toxic to its host's lymphocytes and other immunocompetent cells. This hypothesis is consistent with certain facts known about *F. hepatica* and its relationship with the rat. For example, very young flukes not producing effective quantities of ESP would be expected to be the immunogenic stage of infection, and older, actively feeding flukes would be nonimmunogenic. The early development of resistance after initial infection, the waning resistance in long-standing infections and the lack of resistance after implantation with adult flukes are thus explained. The importance of timing of passive serum transfer is also explained; transfer at the time of challenge, before flukes are producing ESP will be effective, but transfer later, when ESP are being produced in effective quantities, will not confer protection.

Further details of *in vitro* and *in vivo* immune adhesion of cells, particularly eosinophils, to *F. hepatica* will be published elsewhere, and electron microscopic studies of the immune killing of *F. hepatica in vitro* and *in vivo* are in progress.

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Received 5 July; accepted 7 August 1978.

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Membrane noise and conductance increase during single spermatozoon–egg interactions

ALTHOUGH one of the earliest events of fertilisation of the sea urchin egg is a depolarisation and decrease in resistance of the egg plasma membrane^{1–3}, it is not known exactly what change occurs immediately after the interaction with a single spermatozoon. We report here that in *Paracentrotus lividus* this change is a depolarisation step of 1–2 mV, occurring 13 s before the main electrical change. The step is accompanied by an increase in cell noise and a decrease in resistance. In other biological membranes, such an observation has been interpreted as the introduction of ionic channels into the membrane^{4,5}. From this analogy we calculate that the single channels introduced into the egg plasma membrane during fertilisation have a conductance greater than 33 pS.

At sperm densities of 10^7 ml⁻¹ or greater we observed a fertilisation action potential similar to that already reported by Steinhardt *et al.*¹ (their Fig. 3) and Ito and Yoshioka³ (their Figs 2 and 3). In their recordings the depolarisation was composed of two phases. The initial phase has been called the shoulder of the action potential³. Closer observation of our records shows that the shoulder is composed of discrete events.

Progressively reducing the sperm density changed the configuration of the shoulder. For example, at a sperm density of 10^6 ml⁻¹, a shoulder was composed of three or four discrete steps, whereas at densities below 10^5 ml⁻¹ it was composed only of a single step. At very low densities, such as those used in Fig. 1a, it was not uncommon to wait several minutes for a reaction to occur. Both single steps and shoulders lasted for 13 s at 22 °C.

The single step is the minimum detectable event during the shoulder phase of the fertilisation action potential. Eggs displaying the minimum event cleave normally. As sea urchins cleave abnormally if more than one sperm enters the egg, we deduce that a step corresponds to the successful interaction of the fertilising spermatozoon. Eggs which display shoulders cleave abnormally and are polyspermic.

Rothschild and Swann^{6,7} have shown for *Psammochinus miliaris* that at sperm densities of 10^5 ml⁻¹, the number of sperm–egg collisions will be 0.16 s⁻¹. The probability for a successful collision was measured as 0.12, so that on average, the successful encounter rate should be once every 50 s. This supports our interpretation that a step corresponds to one successful encounter.

A typical step and fertilisation action potential is shown in Fig. 1a. Steps were always accompanied by an increase in voltage noise. Lowering the temperature prolonged the step. At 22 °C the average duration was 10.9 ± 1.8 s ($n = 12$), and at 12 °C the average duration was 25.0 ± 4.3 s ($n = 3$). Similarly the rise time of the fertilisation action potential decreased from 1.13 ± 0.54 mV s⁻¹ to 0.27 ± 0.09 mV s⁻¹.

Figure 1b shows a step during an experiment carried out at 5 °C in which the duration was 79 s. The longer step allowed a reasonable time for spectral analysis of the noise increase, and for an estimate of the change in variance before and after the step. This analysis is shown in Fig. 2. The voltage noise during the step increased primarily in the low frequencies. At rest, membrane resistance may range from 4 to 18 KΩ cm² (egg diameter 80 μm); during a step the resistance was always reduced by one-third to one-half.

To obtain an estimate of the elementary conductance change contributing to a step, we calculated the ratio of the change in noise variance to the change in potential. This ratio for Fig. 1b was 10 μV, which may be taken as the size of the elementary event contributing to the noise. Taking the specific resistance of

the egg during the step at its mean value of $6 \text{ K}\Omega \text{ cm}^2$ ($30 \text{ M}\Omega$), the current associated with this elementary event was 0.33 pA . To calculate the conductance through which this current passes, an estimate of the equilibrium potential of the step is required.

Progressively offsetting the resting potential towards zero, using a second intracellular electrode, resulted in a gradual decrease in the size of the step. At -3 mV , a step was difficult to observe and the noise increase was minimal; however the fertilisation reaction still occurred. This experiment suggested an equilibrium potential near zero. It was not possible to invert the step as fertilisation was suppressed when the egg was held positive, as reported elsewhere⁸.

Using the data from Fig. 1b, with a resting potential of 10 mV , the elementary conductance was calculated to be 33 pS . Repeating the calculation for three other examples gave the values 16 , 41 and 92 pS . In none of these cases was the resistance measured simultaneously with the noise. These values are large compared with the conductance of ion-specific channels, for example, Na and K channels in nerve membranes; however, they are similar to nonspecific drug-induced channels^{4,5}.

In the absence of Ca^{2+} a step did not occur; if calcium was added a normal step was observed and development ensued. The step seems to be a general phenomenon in sea urchins, as we have also seen it in *Psammechinus* and *Arbacia*.

What is the relationship between the electrical events constituting a step and the morphological events which occur during fertilisation? Possibilities include labile attachment of the spermatozoon to the vitelline membrane, strong attachment either to the vitelline membrane or egg plasma membrane, or fusion of the two gametes. Also, the acrosomal reaction is

Fig. 1 *a*, Intracellular recording from a mature egg of *Paracentrotus lividus* during fertilisation. Sperm density was 10^3 ml^{-1} . Egg jelly was removed by acid seawater ($\text{pH } 5.0$). The fertilisation action potential is the transient change in membrane potential shown in the latter half of the upper trace. The fertilisation potential is preceded by a step. The upper trace was d.c.-coupled whereas the lower trace, a.c. coupled from 1 Hz , demonstrates the noise increase during the step. (The noise trace saturates during the fast rise of the fertilisation action potential, and is omitted.) Vertical bar represents 12 mV (upper), 1 mV (lower); horizontal bar represents 5 s and also indicates the zero potential level. Temperature was 25°C . *b*, A step at 5°C . Vertical bar represents 6 mV (upper), 1 mV (lower); horizontal bar represents 5 s and indicates zero potential level.

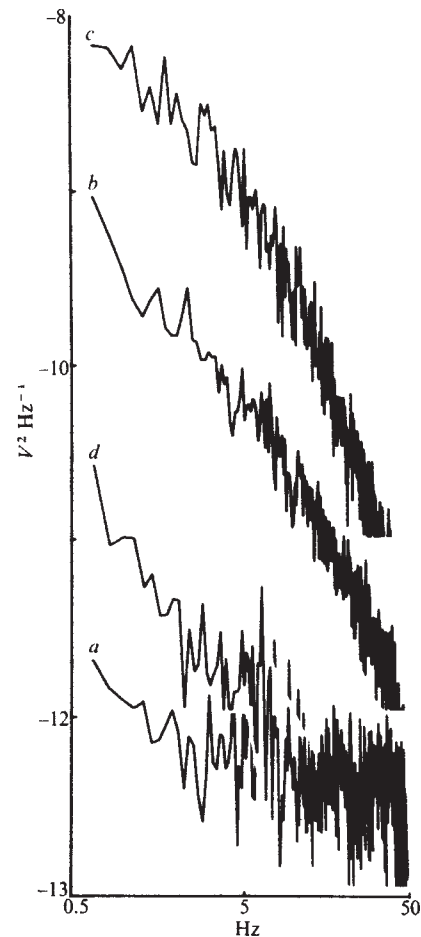
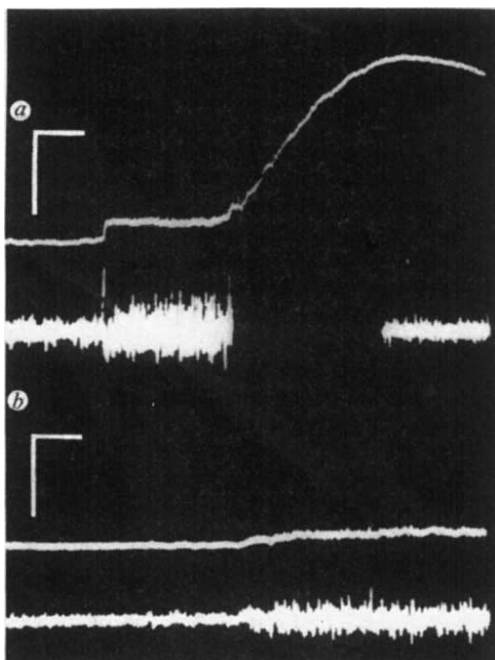


Fig. 2 Power spectral density of voltage noise from the experiment shown in Fig. 1b. *a* microelectrode noise before the experiment; *b*, cell noise before the step; *c*, during the step; *d*, microelectrode noise after pulling out of the egg. About 40 s of data were used to estimate *b* and *c*; only half this amount for *a* and *d*. The microelectrode noise although increased after the experiment was well below the cell noise during the step. The spectra are limited by a.c. coupling at 1 Hz , and the time constant of the membrane.

generally considered as a prerequisite to sperm entry⁹. Aketa and Ohta¹⁰ have shown in *Pseudocentrotus* that the acrosomal reaction occurs following attachment to the vitelline membrane.

Arbacia spermatozoa made labile attachments to *Paracentrotus* eggs, but such attachments were not accompanied by an electrical change. However, at $\text{pH } 9.4$ a normal step and fertilisation action potential occurred qualitatively similar to that recorded for homologous fertilisation of either species. High pH alone did not activate the eggs; however, *Arbacia* sperm exposed to high pH underwent the acrosomal reaction as reported elsewhere¹¹. We deduce from this evidence that the step is subsequent to the acrosomal reaction. This interpretation would also explain the absence of the step in Ca^{2+} free seawater because the acrosomal reaction is Ca^{2+} dependent¹¹.

We suggest that a factor is released from the acrosomal vesicle which causes directly or indirectly the introduction of conductance channels into the egg plasma membrane. Introduction of many conductance channels into a membrane would result in a depolarisation and a noise increase and a decrease in resistance^{4,5}. Although our results do not rule out cell fusion as giving rise to the step, the conductance increase cannot be explained by the introduction of a simple hole into the egg plasma membrane.

We thank A. Monroy for criticism of the manuscript and the Laboratory of Physiology, University of Leiden for computer facilities. This work was supported by the CNR Project on

Biology of Reproduction, EMBO and the Royal Society, London. L.J.D. is on leave from Emory University; V.T. is from the Institute of General Physiology, Naples.

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Received 20 February; accepted 18 July 1978.

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Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity

PHOSPHOLIPIDS are a major component of biomembranes and provide the fluid matrix for protein movement and organisation. Membrane fluidity has been closely related to the biological and biochemical processes such as transport of chemicals, cell fusion, and protein rotation and diffusion^{1,2}. Genetic, nutritional and temperature manipulation have been used to alter the fluidity of the cell membrane^{3,4}, but physiological and biochemical events that regulate membrane fluidity are still unknown. We report here that the enzymatic methylation of phosphatidylethanolamine in erythrocyte membranes induces a marked increase of the bulk membrane fluidity.

An examination of the role of phospholipid methylation in the regulation of membrane fluidity was prompted by the find that two methyltransferases are involved in the conversion of phosphatidylethanolamine to phosphatidylcholine⁵. These enzymes were found to be asymmetrically distributed in the erythrocyte membrane. The first enzyme that converts phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine faces the cytoplasmic side, whereas the second enzyme that adds two additional methyl groups from S-adenosyl-L-methionine to form phosphatidylcholine is located on the external surface of the erythrocyte membrane⁶. The substrate for the first methyltransferase, phosphatidylethanolamine, resides mainly on the cytoplasmic side and the product of the second methyltransferase, phosphatidylcholine, is present largely on the external surface of the membrane^{6,7}. Thus, translocation of phospholipids from the inside to the outside of the membrane is facilitated by a stepwise methylation of phospholipids. The enzymatic methylation of phosphatidylethanolamine to phosphatidylcholine takes place within the membrane, Mg²⁺ playing a critical part⁶. To examine the effect of such phospholipid rearrangement across the membrane on the lipid fluidity, the following experiments were carried out.

Fresh samples of blood were obtained from ether-anaesthetised male Sprague-Dawley rats (150 g) by cardiac puncture using heparin as anticoagulant. The erythrocytes, washed with 0.9% NaCl solution, were haemolysed with 40 volumes 5 mM Tris-phosphate buffer, pH 8.0, containing 1 mM MgCl₂ (ref. 8). The ghosts thus obtained were suspended into 10 volumes 50 mM Tris-glycylglycine buffer, pH 8.0, containing 5 mM MgCl₂. After centrifugation at 4°C, 0.3 ml of packed ghosts were suspended in 0.7 ml of the same buffer, containing various

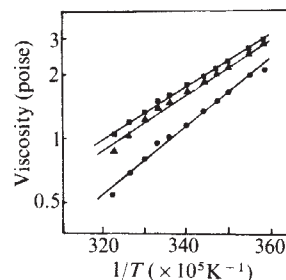


Fig. 1 Effects of incubating erythrocyte ghosts with S-adenosyl-L-methionine and S-adenosyl-L-homocysteine on membrane microviscosity. Erythrocyte cell ghosts were preincubated at 4°C in 5 mM MgCl₂ and 50 mM Tris-glycylglycine buffer, pH 8.0, containing a 50 μM S-adenosyl-L-methionine (●); b, 50 μM S-adenosyl-L-methionine and 2 mM S-adenosyl-L-homocysteine (▲); c, control (■). The ghosts were, then, resealed and labelled with DPH at 37°C for 1 h. The amount of methyl group incorporated into lipids was 9 pmol per mg protein and 0.5 pmol/mg protein in the absence and presence of S-adenosyl-L-homocysteine, respectively. The calculation of the microviscosity at 25°C was made according to the method of Hare *et al.*¹⁰.

concentrations of S-adenosyl-L-methionine and/or S-adenosyl-L-homocysteine, and incubated overnight (approximately 16 h) at 4°C. Before use, the ghosts were washed with 10 volumes of a solution containing 150 mM NaCl, 10 mM Tris-HCl buffer, pH 8.0, and 5 mM MgCl₂ (solution A). The fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was used for the measurement of microviscosity by the method of Shinitzky and Barenholz⁹. Fluorescence labelling was carried out by incubating the red blood cell ghosts in the solution A containing 1 μM DPH at 37°C for 1 h. Labelled cells were washed and suspended in solution A at a concentration of 0.1–0.2 mg cell protein per ml.

The microviscosity of the erythrocyte membrane was found to be a function of temperature but no phase transition was apparent over a temperature range of 6 to 36°C (Fig. 1). S-adenosyl-L-methionine was introduced inside the ghosts by preincubation at 4°C. When the resealed ghosts were labelled with DPH at 37°C for 1 h, the methylation of phospholipids as determined by procedures described previously⁵ took place simultaneously, but no significant methylation was observed at 4°C. When S-adenosyl-L-methionine was present inside the ghosts, the microviscosity markedly decreased (Fig. 1). This reduction was almost completely abolished when S-adenosyl-L-homocysteine was present inside the cells together with S-adenosyl-L-methionine. S-adenosyl-L-homocysteine inhibited the methylation of phosphatidylethanolamine and itself did not affect the membrane microviscosity. These results indicate that

Table 1 Correlation between microviscosity changes and amount of phosphatidyl-N-monomethylethanolamine formed

SAM (μM)	[³ H]-methyl incorporation			Microviscosity (P)
	PME (pmol per mg protein)	PDE (pmol per mg protein)	PC (pmol per mg protein)	
0	—	—	—	1.62
4	1.15	<0.01	<0.01	1.32
12	1.80	0.1	0.1	1.18
25	1.90	0.50	0.40	1.08
50	1.95	1.20	1.50	1.06
100	1.97	2.40	3.60	1.09

SAM, S-adenosyl-L-methionine; PME, phosphatidyl-N-monomethylethanolamine; PDE, phosphatidyl-N,N-dimethylethanolamine; PC, phosphatidylcholine. Varying amounts of S-adenosyl-methyl-³H-L-methionine were introduced into rat erythrocyte ghosts and incubated as described in Fig. 1. The methylated phospholipids formed enzymatically were quantified after separation by thin layer chromatography with the solvent system, *n*-propyl alcohol/propionic acid/chloroform/water (3/2/2/1 v/v)⁵.

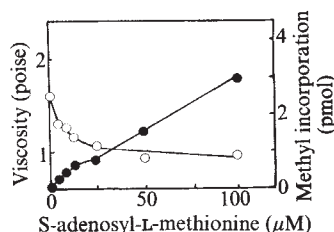


Fig. 2 Effects of various concentrations of *S*-adenosyl-L-methionine on microviscosity. Erythrocyte blood cell ghosts were preincubated at 4°C with various concentrations of *S*-adenosyl-methyl-³H-L-methionine and then labelled with DPH at 37°C to measure microviscosity. A part of the mixture equivalent to 0.2 mg protein was examined for ³H-methyl incorporation into the lipid fractions after extraction with 3 ml of chloroform/methanol/1 M HCl (6/3/1)^{5,6}.

the methylation of phospholipids alters the membrane fluidity.

Previous work has shown that the enzymatic methylation of phosphatidylethanolamine has a low K_m for *S*-adenosyl-L-methionine (1.4 μM), whereas the methylation of phosphatidyl-*N*-monomethylethanolamine to phosphatidylcholine had a high K_m for the methyl donor (0.1 mM)⁵. To determine whether synthesis of phosphatidyl-*N*-monomethylethanolamine and/or phosphatidylcholine was responsible for the changes in fluidity, varying concentrations of *S*-adenosyl-L-methionine were introduced into erythrocyte ghosts. Figure 2 shows the effects of increasing concentration of *S*-adenosyl-L-methionine on membrane viscosity. The concentration of *S*-adenosyl-L-methionine required for half-maximal reduction of the microviscosity was approximately 5 μM. A plot of the amount of methyl group incorporated against *S*-adenosyl-L-methionine concentration shows an inflection point at about 25 μM, indicating the presence of two enzymes in the methylation of phospholipids (Fig. 2). The identity of the methylated phospholipids at various concentrations of *S*-adenosyl-L-methionine were measured by introducing the methyl-³H labelled compound into the erythrocyte ghosts. When the methylated products were isolated and quantified using thin layer chromatography⁵, monomethylated phosphatidylethanolamine was found to form predominantly at concentrations of *S*-adenosyl-L-methionine below 25 μM (Table 1). In these conditions, the membrane microviscosity reached nearly maximum. At concentrations above 25 μM, phosphatidylcholine were increasingly formed (Table 1). These results indicate that the erythrocyte membrane fluidity changes are caused mainly by the enzymatic methylation of phosphatidylethanolamine to phosphatidyl-*N*-monomethylethanolamine. In contrast to phosphatidylethanolamine and phosphatidylcholine, we have found that phosphatidyl-*N*-monomethylethanolamine is deeply embedded in the erythrocyte membrane⁶. It is not susceptible to the digestion by phospholipase C from either the inside or outside erythrocyte ghosts. The accumulation of phosphatidyl-*N*-monomethylethanolamine within the lipid bilayer could account in greater part for changes of membrane fluidity.

Alterations in membrane fluidity due to phospholipid methylation should produce pleiotropic changes in membrane function. Preliminary results already indicate that binding of a catecholamine agonist with the β-adrenoceptor stimulates an increase of phospholipid methylation in reticulocytes and facilitates the lateral mobility and coupling of the receptor with adenylate cyclase (F. H., W. J. Strittmatter and J. A., unpublished).

We thank Drs M. H. Gottlieb and R. Aoshima, NIH, for their help and discussions in measurement of fluorescence.

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Recognition and genetic control of helper determinants for cell surface antigen Thy-1

THE hapten-carrier effect in immune responses demonstrates that one antigenic determinant can strongly modulate the antibody response to a second determinant if the two exist in physical association. This phenomenon, in more general form termed associative recognition¹, has been analysed with soluble antigens and reveals collaboration between carrier-directed, helper T cells and hapten-directed, antibody-producing B cells. In contrast to soluble antigens, antibody responses to surface antigens of nucleated cells are poorly understood and although collaboration among T and B cells is indicated in several studies^{2–4} the antigenic sites recognised by these T cells and the extent of associative recognition among the different antigenic determinants on cell membranes is not known. Thus, it is not clear whether the determinants recognised by T and B cells are controlled by a single gene and are joined covalently or if the two determinants are controlled by independent genes and are only loosely associated or even unassociated on the cell surface. This knowledge would help determine the character and extent of molecular interactions on the surface of nucleated cells as well as principles of immunogenicity of antigens expressed on cell membranes. This report extends previous findings^{1,5} and describes a new experimental approach to study, in primary responses the role of alloantigens in the immunogenicity of Thy-1 through the use of closely related donor-recipient strains and an assay for antibody plaque-forming cells (PFC). The results are consistent with the notion that in primary immune responses Thy-1 is 'helpless' but that in appropriate donor strains, help may be supplied by other alloantigens controlled elsewhere in the donor genome.

To examine the contribution of other alloantigens to the immunogenicity of Thy-1 we immunised AKR/J (Thy-1.1) mice with thymus cells from AKR/Cum or CBA (both Thy-1.2). Since AKR/Cum is closely related to AKR/J^{6,7} differences between the immunogenicity of CBA and AKR/Cum cells could be attributed to alloantigens of CBA donors absent in AKR/Cum cells. CBA thymus cells induced a large anti-Thy-1.2 antibody response in AKR/J mice which was measured as numbers of direct PFC per spleen (Table 1). This response peaked at 7 d while AKR/Cum thymus cells induced few or no PFC at any time. The response is fully specific for Thy-1.1 determined as before⁸ and considered further below. Two possible reasons for this difference in immunogenicity apart from the contributions of other donor alloantigens were considered; first that AKR/Cum thymus cells express much less Thy-1.2 than CBA cells, was excluded in serological tests which showed them to be indistinguishable in the ability to absorb anti-Thy-1.2 antibody, and second that the Thy-1.2 antigen of AKR/Cum is suppressive, is unlikely since mixtures of CBA and AKR/Cum cells are immunogenic (data not shown) and since in a more rigorous control the antigen on (CBA × AKR/Cum)_F₁ hybrid cells is highly immunogenic (Table 1).

The augmentative effect of alloantigens was further explored in other strain combinations and with cells having the reciprocal Thy-1 alloantigen. (CBA × AKR/Cum)_F₁ mice were

immunised with thymus cells from strain AKR, (AKR × AKR/Cum) F_1 or (AKR × CBA) F_1 all of which produced few or no PFC in the recipients (Table 2). However, thymus cells from (AKR × B10.BR) F_1 mice were highly immunogenic. Each of the four donors can be shown to be immunogenic for Thy-1.1 in the appropriate recipients, however in the present experiment only the (AKR × B10.BR) F_1 cells contain numerous alloantigens to which the recipients are not genetically tolerant. Thus a Thy-1 incompatibility is insufficient to produce strong PFC responses and other alloantigens seem to augment Thy-1 immunogenicity. This result moreover demonstrates that in F_1 hybrid antigenic cells, chromosomes from one parent can control determinants which help the response to another antigen controlled by independent chromosomes from the other parent. These findings have been confirmed in a second strain pair (A/Thy-1.1 and A/Thy-1.2) in which large numbers of PFC were induced by A/Thy-1.1 cells in B10.A mice (H-2) compatible but otherwise allogeneic) but very few in congenic A/Thy-1.2 recipients.

A nonimmunological process may be proposed to explain the augmentative effects of an allogeneic haplotype, however this is unlikely. For example, it would be otherwise difficult to explain how the CBA donor haplotype augments the anti-Thy-1.2 response in AKR recipients to (CBA × AKR/Cum) F_1 (Table 1) but the same haplotype fails to augment the anti-Thy-1.1 response to (CBA × AKR) F_1 in (CBA × AKR/Cum) F_1 recipients (Table 2). The immunological basis of the effect is supported by evidence of thymus-dependency described below. Moreover, in a concurrent but more complex study of secondary PFC responses to Thy-1 in which reciprocal specificity controls are possible we have found the recognition of the non-Thy-1 helper determinants to be immunologically specific (E. A. Clark and P.L., unpublished).

In classical hapten-carrier systems it is held that these two antigenic components must be firmly associated for the haptenic determinant to be immunogenic; mixtures of molecules are not active⁹. To test if the Thy-1 determinant and the putative helper determinants need to be physically associated, (CBA × AKR/Cum) F_1 mice were immunised with two cell preparations. The first inoculum comprised (AKR × B10.BR) F_1 thymus cells which express both the B10.BR and the Thy-1.1 alloantigens on

Table 2 Immunogenicity of Thy-1 is helped by other donor alloantigens

Thymus donor	Thy-1 phenotype	Anti-Thy-1.1 PFC per spleen (s.e.m.)
AKR	Thy-1.1/1.1	75 (19)
(AKR × AKR/Cum) F_1	Thy-1.1/1.2	48 (7)
(AKR × CBA) F_1	Thy-1.1/1.2	0 (0)
(AKR × B10.BR) F_1	Thy-1.1/1.2	4,328 (680)

(CBA × AKR/Cum) F_1 mice which were age and sex matched were arranged in groups of five recipients each and were injected i.v. with 4×10^7 of the indicated cell suspension. PFC were determined after 7 d. No difference in immunogenicity of male and female donors was detectable in several controlled experiments and subsequently both donors were used.

each cell and the second was a mixture of B10.BR and AKR cells where the helper alloantigens and the Thy-1.1 determinants are on separate cells. It is evident from the results in Table 3 that the helper alloantigens must be expressed on the cells which carry the Thy-1 determinant for augmentation to result. Mixtures of cells fail to show any premium effect. This indicates that the Thy-1 antigenic determinant must be associated with the helper determinant in the same cell membrane for effective presentation to the immune system. It is unknown if this physical association of helper and Thy-1 represents linkage at the molecular level or if they are separate but joined by a membrane bridge. This observation differs fundamentally from the properties of helper sites controlled by the I-region which augment the induction of cytotoxic T cells¹⁰ in which help results, even when the two classes of antigen are expressed on separate cells. Taken together these studies indicate two pathways of antigen processing for T help to cellular antigens which are distinguished in their requirements for some form of structural association of the helper site and the determinant which is helped.

Stronger evidence for the immunological basis of the augmentation rendered by donor alloantigens was obtained by testing if this effect is thymus-dependent. From the results using 'B' mice (Table 3) it is apparent that this form of augmentation is thymus-dependent. The reduced overall magnitude of response is attributed to irradiation effects. The data also show that all the PFC detected on target cells of AKR produce specific anti-Thy-1.1 antibody since AKR/Cum target cells show no PFC.

Inbred strains of mice may differ for a multitude of alloantigens and those which are expressed on thymus cells are candidate helper determinants. Since it is known that structural associations between products of different genes can occur on the cell surface and that these intermolecular interactions are highly specific, for example between β_2 -microglobulin and H-2 or TL¹¹, it was possible therefore that help for Thy-1 was similarly associated with one or only a few select donor alloantigens controlled by their respective genes. To estimate the number of these genes, that is, of independently segregating genetic loci controlling the helper effect for Thy-1, a backcross experiment was done. A homogeneous population of AKR recipient mice was arranged into groups of two or three mice each and each group was inoculated with the thymus cells from individual (CBA × AKR/Cum) F_1 × AKR/Cum backcross progeny.

Since the F_1 hybrids are highly immunogenic it was expected that segregation of the helper loci of CBA would occur upon backcross mating the F_1 to the poorly immunogenic parent. Thymus cells from 76 backcross progeny mice of three weeks of age were injected into AKR recipients. Other recipients were injected with thymus cells from AKR/Cum and (CBA × AKR/Cum) F_1 donors which served as negative and positive controls respectively. Thus all donor mice were homozygous for the Thy-1.2 determining allele and segregants differed in their expression of putative helper alloantigens from the CBA parent.

Table 1 Weak primary response of AKR to Thy-1.2 alloantigen on closely related donor cells

Dose	Day	Donor strain/PFC per spleen (s.e.m.)		
		CBA(Thy-1.2)	AKR/Cum(Thy-1.2)	
4×10^7	3	39 (26)	0 (0)	
4×10^7	5	519 (237)	21 (19)	
4×10^7	7	2241 (252)	15 (8)	
4×10^7	9	270 (52)	90 (60)	
4×10^7	11	39 (10)	51 (18)	
		CBA	AKR/Cum	(CBA × AKR/Cum) F_1
8×10^7	7	3429 (861)	0 (0)	2,100 (542)
4×10^7	7	2,241 (252)	15 (9)	1,221 (258)
2×10^7	7	1269 (510)	30 (30)	909 (441)

Homogeneous pools of AKR mice (all mice were bred by ICRF) were arranged in groups of four and inoculated on the appropriate days i.v. with 4×10^7 donor thymus cells which has been washed three times in Eagle's minimal essential medium (MEM). Spleens were removed from all the recipients at one time and were individually dispersed with a loose fitting homogeniser in 10 ml of MEM supplemented with 10% heat-treated (56 °C, 30 min) newborn calf serum (CA₁₀). The spleen cells were centrifuged (800 r.p.m. for 10 min) and resuspended in 1.5 ml of MEM. Thymus cells were obtained from CBA mice aged 3 weeks, washed three times as above and resuspended at 10% v/v in MEM-CA₁₀. 50 μ l samples of the spleen cell suspensions were mixed with 20 μ l of the target thymus cells in 2 ml capacity plastic tubes at 37 °C and 180 μ l of 0.95% Agarose in MEM-CA₁₀ at 45 °C were added. The cell suspension was applied directly to a 35 mm plastic Petri dish, incubated, treated with complement and stained as described⁸.

The results in Fig. 1 show that the backcross progeny distribute into two categories of immunogenicity, those which fall into the zone of the poorly immunogenic AKR/Cum donors and those well above this zone and are thus augmented. Of 76 backcross mice, 23.7% (18 mice) were of the low immunogenic type and 76.3% (58 mice) were augmented immunogens as compared with controls. This result is statistically consistent with the presence of two unlinked chromosomal loci in the donor CBA genome, each capable of augmenting in antigenic thymus cells the immunogenicity of Thy-1.2 for primary antibody responses (chi-square test $0.95 < P < 0.90$). The scattergram also suggests that the two 'helper loci' may differ in strength and that their representative genes are fully penetrant and dominant or codominant. It is unknown if the determinants which help the Thy-1 response are exclusive in their effect to Thy-1 or if they can also help other surface antigens. The identification of the helper determinants and their properties is therefore important to pursue.

There are indications that similar associative recognition phenomena may apply to responses to other surface antigens. In a recent study it was shown that, even subsequent to extraction and purification, a potent carrier effect of one cell surface molecule for another with which it natively associates on the cell membrane is preserved¹². The requirement for help of one surface antigen from other antigens may explain why most congenic immunisation regimens fail to produce anti-Ly antibodies¹³ as well as the absence of IgG production in a congenic H-2 immunisation¹⁴. In successful congenic immunisations the response may be more thymus-independent^{2,3} or alternatively obtain T cell help from antigens coded for by closely-linked genes. There is also evidence that some helper determinants may themselves be latent and are immunogenic only in the presence of other antigens¹⁵.

In summary the results show that the Thy-1 antigen is much more immunogenic if presented on donor cells which also carry other alloantigens and that this helper effect is thymus-dependent. These helper sites and the Thy-1 antigen must be expressed on the same cell surface and not on separate cells, indicating

Table 3 Evidence that helper alloantigens must be on the cells expressing the determinants which are helped and thymus-dependence of the effect

Expt 1	Thymus donor	Thy-1 phenotype	Anti-Thy-1.1 PFC per spleen (s.e.m.)
	AKR	Thy-1.1/1.1	345 (127)
	B10.BR	Thy-1.2/1.2	0 (0)
	(AKR × B10.BR)F ₁	Thy-1.1/1.2	4,554 (425)
	AKR + B10.BR ^a	Thy-1.1/1.1 + Thy-1.2/1.2	387 (72)
	AKR + B10.BR ^b	Thy-1.1/1.1 + Thy-1.2/1.2	186 (36)
Expt 2	Thymus donor	Pre-irradiation treatment	Anti-Thy-1.1 PFC per spleen (s.e.m.)
	AKR + B10.BR	Thymectomy	54 (71)
	(AKR × B10.BR)F ₁	Thymectomy	9 (20)
	AKR + B10.BR	Sham thymectomy	33 (22)
	(AKR × B10.BR)F ₁	Sham thymectomy	*753 (252)

(AKR/Cum × CBA)F₁ mice, in groups of five recipients each, were injected i.v. with 4×10^7 of the indicated cell suspension except for the cell mixture where in (a) 8×10^7 cells were injected and in (b) 4×10^7 cells were injected. Cells were mixed in a 1:1 ratio. PFC were determined after 7 d. In Experiment 2 (CBA × AKR/Cum)F₁ mice were thymectomised or manipulated surgically but not thymectomised. After a recovery interval of 3 weeks all mice were given 750 rad of γ -irradiation and 5×10^6 fetal liver cells from syngeneic mice. The mice were used after an additional 5-week period.

* Portions of these spleens (1/30) were tested on AKR/Cum target and no PFC were detectable.

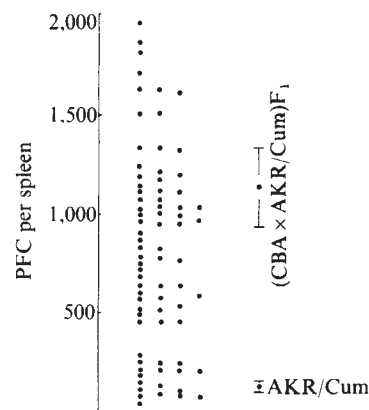


Fig. 1 Distribution of immunogenicity of thymus cells from individual backcross mice with segregating helper determinants. Evidence for two antigenic 'helper genes'. A large age-matched pool of AKR female mice was randomised and arranged in groups of two or three recipients per backcross donor thymus and in groups of five or six for control thymus suspensions (a pool of several donors). The recipients were immunised with 4×10^7 (CBA × AKR/Cum)F₁ × AKR/Cum backcross thymus cells i.v. and PFC per spleen determined on day 7. Each point represents the mean PFC per spleen of two or three recipients. Vertical bars represent ± 1 s.e.m.

that some form of physical bridge between Thy-1 and its helper is important in the presentation of the surface antigen to the immune system similar to hapten-carrier antigens. Further, we have shown that highly effective helper sites are controlled by chromosomes lacking the relevant Thy-1 antigen and the data suggest that only two genetic loci control these helpers in primary antibody responses.

We thank Professor N. A. Mitchison, and Drs M. Baltz and P. Golstein for discussions and J. Bromberg for reconstituted mice. P.L. was supported by the MRC of Canada and an ICRF Fellowship and T.D. by the Basel Institute for Immunology and the Medical Genetics Center.

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Received 24 April; accepted 4 August 1978.

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Adenylate cyclase in a fibroblast mutant defective in glycolipid and glycoprotein synthesis

THERE is currently wide interest in the biological role of glycolipids and glycoproteins of the cell membrane. As some transformed cells have reduced levels of the more complex gangliosides and neutral glycolipids (see refs 1–3 for reviews), it has been suggested that complex gangliosides and particularly ganglioside G_{M1} may have an important role in regulating cell growth and in the responsiveness of adenylate cyclase (ATP pyrophosphate-lyase (cyclising), EC 4.6.1.1) to regulatory factors^{3,4}. The ganglioside G_{M1} is thought to be required for the activation of plasma membrane adenylate cyclase by cholera toxin; the toxin activates adenylate cyclase⁵ and binds specifically to ganglioside G_{M1} (refs 6–8). To investigate the effect of altered glycolipid and glycoprotein composition on adenylate cyclase activity we have used cell line AD6, a mutant of BALB/c 3T3 cells that is deficient in glucosamine phosphate acetyl transferase^{9–12}, and therefore partially blocked in its ability to synthesise complex carbohydrates, glycoproteins and glycolipids. Our results show that AD6 cells have a decreased responsiveness to cholera toxin, indicative of an altered membrane ganglioside composition, yet the responses of the enzyme to GTP, adrenaline, prostaglandin E_1 and fluoride are unaltered. Our results indicate that decreased synthesis of complex gangliosides does not result in alterations in adenylate cyclase other than a decreased response to cholera toxin.

For the present studies cells were grown in Dulbecco–Vogts' modified Eagle's medium supplemented with 10% calf serum (Colorado Serum) as described by Pouyssegur and Pastan⁹. For the determination of cholera toxin activation of adenylate cyclase cells were changed to serum-free medium 12 h before addition of cholera toxin. Cells were then incubated with the indicated concentration of cholera toxin (Schwarz-Mann) for 3 h

Fig. 1 Effect of cholera toxin treatment of intact Swiss 3T3 (●), BALB/c 3T3 (△), SV40-BALB (○), or AD6 (▲) cells on adenylate cyclase activity measured in a crude membrane fraction prepared from the indicated cell type. Cells were changed to serum-free Dulbecco–Vogt modified Eagle's medium and incubated with the indicated concentration of cholera toxin for 3 h before determination of adenylate cyclase activity. Enzyme activity was measured as described¹³ with 0.2 mM ATP, 3 mM Mg^{2+} and 1 mM EGTA.

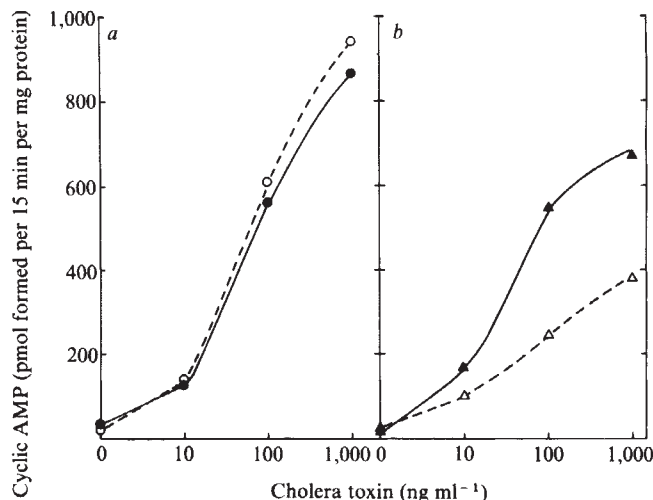
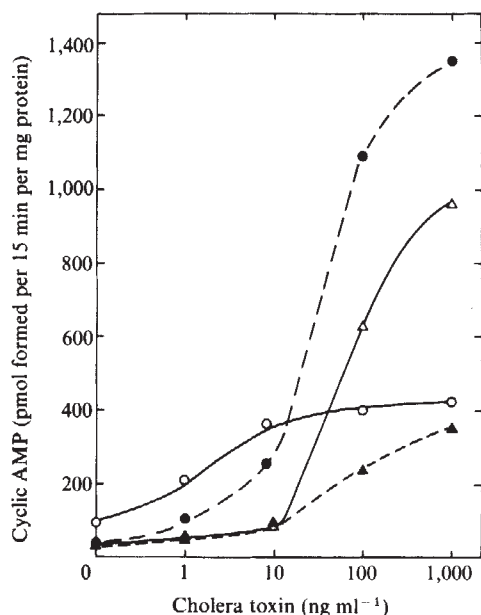


Fig. 2 Effect of G_{M1} on cholera toxin activation of adenylate cyclase in wild-type BALB/c 3T3 (a) or AD6 cells (b). Cells were incubated for 18 h in serum-free medium with (closed symbols) or without (open symbols) 50 pmol G_{M1} per dish, then washed three times with phosphate-buffered saline and once with serum-free growth media. Cells were then incubated in serum-free media with the indicated concentration of cholera toxin for 3 h before determination of adenylate cyclase activity as described in Fig. 1.

before collection. A crude membrane fraction was prepared and adenylate cyclase activity measured as described elsewhere¹³. Triplicate determinations of adenylate cyclase activity were routinely within $\pm 10\%$.

The block in the formation of *N*-acetylglucosamine and derived amino sugars would be expected to lead to decreased synthesis of the more complex gangliosides from ganglioside G_{M3} (see the pathway for ganglioside biosynthesis presented in ref. 3) as well as glycoproteins. From published data^{14,15} it is established that Swiss 3T3 cells have higher levels of the complex gangliosides than do BALB/c 3T3 cells, and SV40-transformed cells have very low or undetectable amounts of G_{M1} and G_{D1a} . Data in Fig. 1 show that incubation of intact cells for 3 h with cholera toxin markedly enhances basal adenylate cyclase activity. Swiss 3T3 cells which contain more of the complex gangliosides are more sensitive to cholera toxin activation presumably due to G_{M1} functioning as the toxin receptor. AD6 cells have a reduced response to cholera toxin. This result suggests that AD6 ganglioside levels are comparable to the SV40-transformed cells.

A chemically transformed line of mouse fibroblasts has been described which lacked G_{M1} and was unresponsive to cholera toxin (ref. 16). When incubated in medium containing G_{M1} , these cells bound the ganglioside and responded to cholera toxin as measured by activation of adenylate cyclase and elevation of cellular cyclic AMP content^{16,17}. To determine if the incorporation of G_{M1} into AD6 or wild-type BALB/c 3T3 cells would improve the response to cholera toxin, confluent cells were changed to serum-free growth medium and incubated with 50 or 500 pmol G_{M1} per dish (100 mm dish containing 5 ml growth media) for 12 h. The ability of cholera toxin to enhance adenylate cyclase activity was determined as described in Fig. 2. A few of the cells (<15%) detached from the substratum during incubation in serum-free medium; only those cells remaining on the dish were used to determine cholera toxin responsiveness. When AD6 cells were treated with 50 or 500 pmol G_{M1} per dish, a significant increase in cholera toxin activation was observed (Fig. 2). Thus, the incorporation of G_{M1} into the mutant membrane corrects the inability of cholera toxin to activate adenylate cyclase of the mutant to the same extent as observed with the wild-type parent. Similar treatment of the BALB/c 3T3 parent did not result in further stimulation by cholera toxin.

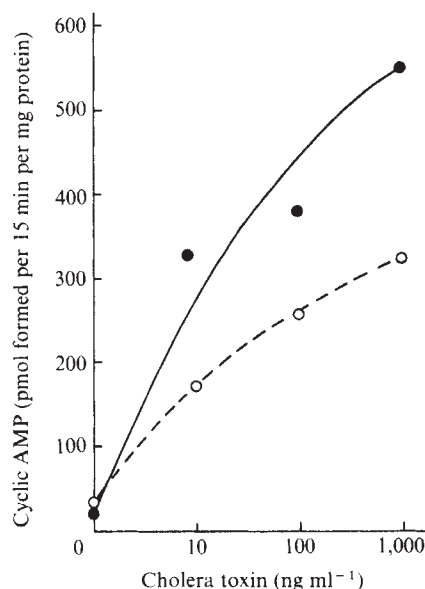


Fig. 3 Effect of *N*-acetylglucosamine on cholera toxin activation of adenylate cyclase in AD6 cells. Cells were grown to confluence in the presence (●) or absence (○) of 10 mM *N*-acetylglucosamine. Addition of the indicated concentration of cholera toxin and determination of enzyme activity were carried out as described in Fig. 1.

Keenan *et al.*¹⁸ have suggested that artificially supplied gangliosides assume a configuration or localisation in the membrane different from that of the native ganglioside. Nevertheless, the results of Fig. 2 show that sufficient ganglioside G_{M1} molecules become associated with the cell surface to serve as receptors for cholera toxin. In addition to showing that ganglioside G_{M1} may act as a cholera toxin receptor, these results emphasise that the low cholera toxin response observed in AD6 cells is due to a decreased ability to recognise and bind cholera toxin.

Previous studies have demonstrated that when AD6 cells are grown with *N*-acetylglucosamine the defect in the *N*-acetylation of glucosamine-6-phosphate is bypassed and the mutant cells revert to the wild-type phenotype^{10,11}. When AD6 cells were grown with 10 mM *N*-acetylglucosamine, the ability of cholera toxin to stimulate adenylate cyclase was enhanced (Fig. 3). This result, together with the ability of exogenous G_{M1} to increase cholera toxin activation of adenylate cyclase in AD6, indicate that it is the altered carbohydrate composition of the plasma membrane of AD6 cells that accounts for their decreased sensitivity to cholera toxin. As AD6 cells have normal growth

properties, this result also indicates that the decreased ganglioside content found in some transformed cells is not by itself a primary cause of the transformed state.

In studies with several virally transformed fibroblast lines a defective adenylate cyclase system has been observed^{19,20}. It is evident from the results presented in Table 1 that BALB 3T3 cells transformed by either the Kirsten sarcoma virus or SV40 virus have elevated basal, GTP-stimulated and fluoride-stimulated adenylate cyclase activities and a decreased or absent response to hormonal activation. Yet, with AD6 cells the adenylate cyclase activity was fully responsive to hormonal stimulation. This result tends to suggest that changes in membrane structure other than modification of the carbohydrate moieties are responsible for the altered properties of adenylate cyclase noted with transformation.

The mutant cell AD6 used in this study has also been found to have decreased binding of epidermal growth factor, presumably because its receptor is a glycoprotein²¹. The use of mutants with defined defects such as AD6 should help elucidate the role of glycolipids, glycoproteins and other complex molecules in cell behaviour.

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Received 7 April; accepted 17 July 1978.

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Table 1 Adenylate cyclase activities of wild-type, AD6 and transformed BALB/c 3T3 cells

Addition	Wild-type BALB/c 3T3	Adenylate cyclase activity		
		AD6	Kirsten- BALB	SV40- BALB
	cyclic AMP (pmol formed per 10 min per mg protein)			
None	31 ± 4	25 ± 3	92 ± 6	101 ± 6
GTP	35 ± 4	29 ± 4	272 ± 9	319 ± 15
GTP + L-adrenaline	378 ± 17	336 ± 20	269 ± 14	346 ± 16
GTP + PGE ₁	89 ± 7	69 ± 7	295 ± 21	352 ± 29
Fluoride	521 ± 27	452 ± 31	1052 ± 46	1138 ± 22

Adenylate cyclase activity was measured as described elsewhere¹³ with 0.2 mM ATP, 3 mM MgCl₂, 1 mM EGTA, and 10 μM GTP, 10 μM L-adrenaline, 10 μg ml⁻¹ prostaglandin E₁ or 10 mM NaF as indicated. A crude membrane preparation of each cell type was used as the source of enzyme. Values presented are the mean ± S.E. for duplicate and triplicate determinations.

Release of acetylcholinesterase from rat hemidiaphragm preparations stimulated through the phrenic nerve

ACETYLCHOLINESTERASE (AChE; EC 3.1.1.7) is known to be transported rapidly within peripheral motor nerves, both towards and away from nerve cell bodies^{1–3}. Of the total amount of neural AChE, approximately 15% is mobile, with about 10% moving towards the nerve terminals and about 5% returning towards cell bodies, the remaining 85% of the enzyme being stationary. Since the mean velocity of orthograde transport is about twice that of the retrograde flow, the total flux of enzyme is 3–4 times greater in the distal than in the proximal direction. Although the fate of this transported

AChE is not entirely clear, it has recently been shown that some of the distally transported enzyme may never arrive at the nerve terminals, being incorporated into the axolemma at sites all along the neurone^{4,5}. This axolemmal incorporation is slow, and it seems likely that AChE would accumulate in the terminals unless there were local mechanisms for its removal, such as proteolytic digestion or extrusion from the cell as has been demonstrated in non-neuronal tissue^{6,7}. We now offer evidence that AChE is released from nerve terminals in response to nerve stimulation and suggest that this release may be parallel to, but separate from, release of neurotransmitter.

Isolated rat phrenic nerve-hemidiaphragm preparations were incubated at 37°C in beakers each containing 1 ml of oxygenated physiological saline solution with (+)-tubocurarine (1.4×10^{-5} M) added to prevent muscle contraction. Preparations were equilibrated in this solution for 1 h, followed by three 15-min incubation periods. At the end of each incubation period, preparations were transferred to new beakers containing fresh medium. The media exposed to tissue during experimental periods were chilled to -20°C immediately after collection and were subsequently analysed for their AChE content by the radiometric assay of Potter⁸. Resting release of AChE was determined from the enzyme activity in the media collected after the first and second experimental periods. This activity averaged 39.2 ± 1.5 nmol of acetylcholine (ACh) hydrolysed per h per ml of medium (mean $\pm$ s.e.m., $n=98$). During the third experimental period, the phrenic nerve was gently lifted out of the bathing medium and stimulated through platinum electrodes with supramaximal 5-V square-wave pulses of 0.4 ms duration and at frequencies of 0–200 Hz. The nerve was positioned such that the oxygenation of the bath splashed medium on the nerve and kept it moist during the stimulation period. To determine the effects of stimulation, resting release (mean activity collected during the first two experimental periods) was subtracted from the activity accumulating during the third period of each experiment. Release from preparations not stimulated during the third period was essentially identical to resting release, whereas stimulation increased the release of AChE in a frequency-related manner (Fig. 1). Stimulation-induced release was statistically significant at 0.2 Hz and highly significant at frequencies of 2 Hz or above, even though the maximum release (at 200 Hz) was only 32% above basal levels (Fig. 1). Since the concentration of (+)-tubocurarine used in these experiments

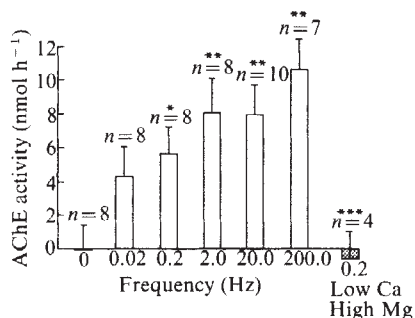


Fig. 1 Release of AChE from nerve-stimulated rat phrenic nerve-hemidiaphragm preparations. Tissues were incubated in isotonic buffer of the following composition (mM): NaCl, 115.0; KCl, 5.0; CaCl_2 , 2.5; MgCl_2 , 0.5; glucose, 5.0; sucrose, 24.0; HEPES (pH 7.4), 15.0; bovine serum albumin, 0.2%. Low Ca^{2+} -high Mg^{2+} buffer differed in the following constituents: CaCl_2 , 0; MgCl_2 , 10.0; sucrose, 3.0. Accumulations of AChE in response to nerve stimulation represent activity in excess of the resting release of enzyme (see text). Mean values and s.e.m. are shown. Significance levels, determined by Student's *t*-test, are: * $P < 0.05$ at 0 Hz; ** $P < 0.005$ at 0.2 Hz; *** $P < 0.001$ at 0.2 Hz in normal buffer. A least squares regression analysis showed a significant linear relationship between release of AChE and log frequency over the range 0.02–200 Hz (slope = 1.46 ± 0.58 , $P < 0.02$).

totally prevented muscle contractions, and has been shown to eliminate muscle action potentials in response to nerve stimulation⁹, it is unlikely that the AChE released from our stimulated preparations arose from the muscle. In two experiments in which we incubated 2-cm lengths of phrenic nerve from which the diaphragm had been removed by a cut close to the muscle, we could detect neither resting release nor release in response to 20-Hz stimulation. We infer from these data that little or no AChE was extruded from non-terminal axons.

Low Ca^{2+} -high Mg^{2+} solutions have been shown to inhibit nerve-stimulated release of the neurotransmitter ACh^{10,11}. In our experiments, resting release of AChE was somewhat reduced in the presence of low Ca^{2+} and high Mg^{2+} (mean = 18.1 ± 1.7) and nerve-stimulated release of AChE at a frequency of 0.2 Hz was completely inhibited (Fig. 1). Although these results suggest a similarity between ACh release and AChE release, there is one important difference. The latter was found to be frequency related up to 200 Hz, whereas ACh release has been reported not to be related to frequency beyond 25 Hz¹². It therefore seems unlikely that the enzyme is extruded from the same sites or by the same mechanism as the neurotransmitter.

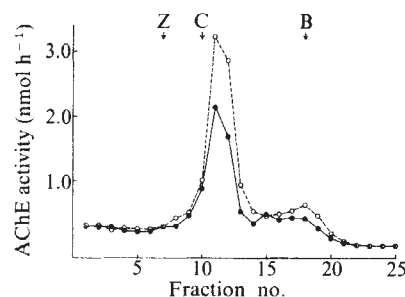


Fig. 2 Velocity sedimentation profile of AChE activity from phrenic nerve-hemidiaphragm effluent. The bathing media of nerve-muscle preparations were layered on 4.8 ml density gradients of 10–40% (w/v) sucrose containing 1 M NaCl, 0.2 mM EDTA, 50 mM Tris-HCl (pH 7.4) and 1% Triton X-100, and were centrifuged at 58,000 r.p.m. for 16 h in an SW65 rotor in an L2 65B ultracentrifuge. AChE activity from resting (●) and 2.0 Hz nerve-stimulated (○) preparations was assayed as described and corrected for effects of the sucrose. Marker proteins used to calibrate the gradients were 16S β -galactosidase (Z); 11.2S catalase (C); and 4.4S bovine serum albumin (B). AChE activity in the 10S (fractions 9–13) and 4S (fractions 16–20) peaks of nerve-stimulated preparations differed significantly ($P < 0.05$) from the corresponding peaks in the resting state as determined by a paired *t*-test. The values represent the means of four nerve-muscle preparations for both resting and nerve-stimulated tissues.

Three isozymes of AChE have been identified in mammalian muscle and nerve, having sedimentation coefficients of approximately 4S, 10S and 16S (refs 13–15). We studied the molecular forms of AChE released into the bathing medium by resting and stimulated nerve-muscle preparations. Tissues were incubated as described above; however, to concentrate the enzyme, the volume was reduced to 0.5 ml and the collection periods were increased to 30 min. Aliquots (0.2 ml) of bathing medium were layered on to sucrose density gradients, centrifuged, fractionated from the bottom of the tube, and assayed for AChE (Fig. 2). The media collected from resting preparations were found to contain both 4S and 10S enzyme, but about three times more of the latter than of the former. Both forms of AChE were released by stimulation, but more than 80% of the released enzyme sedimented at 10S. No 16S AChE was detected in media from resting or stimulated preparations.

The distribution of molecular forms of AChE released into the bathing media from our nerve-muscle preparations does not resemble the distribution in muscle, which contains significant amounts of 16S enzyme, primarily in the endplate

regions, and approximately equal amounts of 10S and 4S enzyme^{13,14}. The distribution of released forms of AChE is, however, similar to the distribution in nervous tissue^{13,15} and resembles especially closely the distribution of forms carried distally by rapid transport along peripheral nerve axons¹⁵. These results suggest not only that the AChE released following nerve stimulation derives from the nerve terminal, but that much of the resting release of AChE also comes from nerves rather than from muscle.

Several investigators have demonstrated that substances other than neurotransmitters may be released from motor nerves on electrical stimulation. Amino acids¹⁶, as well as proteins^{17,18} presumably transported distally from cell bodies, are released from nerve terminals. Although AChE has not previously been identified as being released from nerve-muscle preparations, it has been shown to be released from cultured chick embryo spinal cord cells¹⁹ and from adrenal glands bathed in high potassium solutions^{20,21}. In the latter case, the release was shown to depend on calcium and was believed to arise, at least partly, from splanchnic nerves²¹.

The content of AChE in muscle is influenced, in part, by the motor neurone²²⁻²⁴. This has been demonstrated convincingly by the reduction of AChE activity in denervated muscle. Although denervated muscle is still capable of synthesising AChE, the enzyme levels never reach those of innervated controls²⁵. In addition, blockade of axonal transport in motor nerves with colchicine has produced denervation-like changes in the AChE content of muscles without interrupting neuromuscular transmission²³. These results suggest that muscle AChE is partially regulated by substances that are transported down the nerve and are released from the nerve terminals.

Our results do not exclude the possibility that one of these regulatory substances is AChE itself. The enzyme has not been demonstrated to be associated with synaptic vesicles, but has been shown to be localised in cisternae of smooth endoplasmic reticulum (SER) in nerves²¹. We suggest that these cisternae may release their contents when action potentials invade the nerve terminals. The mechanism of this release could share features in common with the exocytotic release of neurotransmitter from the synaptic vesicles. In any case, if the released AChE were able to diffuse across the synaptic cleft, it might be responsible for some of the trophic effects of nerves on muscle and could even serve as one source of muscle AChE.

This research was supported in part by a grant from the NIH. K.A.S. is a recipient of a National Research Service Award, and S.B. of a Career Development Award, from the NIH.

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Received 4 May; accepted 12 July 1978

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Evidence for two separate opiate peptide neuronal systems

WHEN Hughes *et al.*¹ first described the sequences of the enkephalins, they pointed out that methionine-enkephalin (met-enkephalin) could be found in β -lipotropin (β -LPH 61-65). The entire C-terminal fragment of β -LPH was subsequently found to have opiate-like properties (β -LPH 61-91 or β -endorphin)²⁻⁷, and it seemed reasonable to hypothesise that the primary biosynthetic route for met-enkephalin was β -LPH to β -endorphin and eventually to met-enkephalin. Although this hypothesis awaits biochemical evidence, immunocytochemical studies of these peptides may shed light on the question. If β -LPH and β -endorphin are, in fact, enkephalin precursors which are stored by enkephalinergic neurones, one would expect them to be visualised in met-enkephalin-positive cells. Several laboratories produced immunocytochemical maps of the distribution of enkephalin-like-immunoreactivity (enkephalin-reactivity) in mammalian brain⁸⁻¹¹. Watson *et al.*¹² demonstrated β -LPH-reactive cells and processes in rat brain. This β -LPH reactivity has the same distribution as β -endorphin-like-immunoreactivity (β -endorphin-reactivity) as reported by Bloom *et al.*¹³ and Watson *et al.*¹⁴. However, the distribution of the β -LPH/ β -endorphin-reactive system on the one hand and the met-enkephalin-reactive system on the other seemed quite different. Met-enkephalin-reactivity was seen in many local cell groups throughout the neuraxis, especially the lower brain stem⁸⁻¹¹, whereas β -LPH- and β -endorphin-reactivities were found only in a single hypothalamic cell group with long ascending and descending processes¹²⁻¹⁴. We present here biochemical and anatomical evidence for the existence of two separate opioid systems in brain—an enkephalin system and a β -LPH/ β -endorphin system. We also present immunocytochemical data for the coexistence in the brain of ACTH with β -LPH/ β -endorphin.

The investigation of the coexistence of ACTH with β -LPH is based on evidence from the pituitary where β -LPH and β -endorphin have been localised in the corticotrophs^{15,16}, in fact, within the same granules¹⁷. These two pituitary peptides possess a common precursor of molecular weight, 31,000 as demonstrated in cell cultures¹⁸, and, in the intact rat, are co-released into the plasma upon stress¹⁹. Such evidence, coupled with the biochemical and immunocytochemical demonstration in brain of β -LPH (refs 12, 20, 21) and of ACTH (refs 22, 23), has led us to investigate whether the same relationship between ACTH and β -LPH/ β -endorphin exists in brain as it does in the pituitary.

Because the present studies depend primarily on the use of multiple antibodies with unique cross-reactivities, the following is a summary of their characteristics. Four antisera were used: anti-methionine-enkephalin (our laboratory), anti- β -LPH (from C. H. Li) anti-ACTH and anti- β -endorphin (R. Mains/B. Eipper). Cross-reactivity of these antisera was tested both in radioimmunoassay (RIA) and immunocytochemical systems by classical competition methods. The met-enkephalin antibody has been described elsewhere²⁴. It exhibits no cross-reactivity with β -LPH, β -endorphin, ACTH or β -melanocyte stimulating hormone (β -MSH). Its cross-reactivity with leu-enkephalin is less than 1:200 and with α -endorphin less than 1:250. ACTH antibody was affinity-purified using ACTH 1-24 (ref. 25) and has been shown to be directed against ACTH 11-24. It does not cross-react with β -LPH, β -endorphin, or

their smaller fragments (β -MSH and met-enkephalin). β -Endorphin antiserum is directed against α -endorphin (β -LPH 61–76). It cross-reacts with β -endorphin and with the full β -LPH molecule on an equimolar basis. It does not, however, cross-react with met-enkephalin, ACTH or β -MSH. β -LPH antiserum seems to be primarily directed at the N-terminus of the β -LPH molecule, as it recognises β -LPH (1–41) and does not recognise β -MSH (β -LPH 41–58)^{26,27}. In the conditions of our RIA this antiserum exhibits very low cross-reactivity with β -endorphin (less than 1:100) with a very shallow dilution curve. In fact, we cannot obtain more than 10% inhibition of binding with concentrations of β -endorphin at 100 nM, whereas β -LPH causes 10% inhibition at 0.05 nM. We have carried out extractions of rat pituitary and brain which are optimal for β -LPH with little contamination with β -endorphin. The β -LPH antiserum demonstrates activity against these ' β -LPH extracts'. The dilution curves are not parallel to those of β -endorphin in this RIA, suggesting that we are not measuring the C-fragment of β -LPH, but possibly its N-terminus. It should be noted, however, that β -LPH N-terminus varies with phylogeny and has not been sequenced in the rat.

Finally, some of the immunocytochemical studies using β -LPH antiserum were carried out in the presence of excess β -endorphin (1 μ M) and the results were unaltered. In contrast, the addition in much lower concentrations of β -LPH (5 nM) led to complete blockade of the anatomical demonstration. The low cross-reactivity of this antiserum with β -endorphin, the different slopes of dilution curves and the lack of blockade by β -endorphin in anatomical studies suggest that we are looking primarily at the N-terminus of β -LPH, and that the cross-reactivity with C-fragment is due to a small subpopulation of antibody.

Thus, three of the antisera used in our studies apparently recognise three different aspects of the precursor molecule; that is, ACTH, the N-terminus of β -LPH, and β -endorphin (or C-terminus of β -LPH). A positive response of all three within the same system might indicate the existence within that system of either precursor or of its individual components.

The evidence for two separable opioid systems is derived from both anatomical observations and a lesion study. As mentioned above, only one hypothalamic cell group combines β -LPH and β -endorphin reactivity, whereas met-enkephalin-positive cells can be visualised in several regions throughout the brain stem but not in the β -LPH/ β -endorphin-containing hypothalamic group. Furthermore, some brain areas are rich in met-enkephalin axons and terminals (for example, globus pallidus), but are almost totally devoid of β -LPH/ β -endorphin activity.

To ascertain that these differential distributions are not due to any artefact of visualisation, we have devised a technique for extracting β -LPH and the enkephalins from the same tissue^{21,28}. As the structure of β -LPH in the rat is unknown, and as our extract dilution curves differ in slope from the human β -LPH curve, all values are expressed in units as described in Table 1. Using the β -LPH and met-enkephalin RIAs, we obtain very different distributions of the two peptides in various brain regions. Again, the striatum has the highest concentration of met-enkephalin in the brain (1,750 pmoles per g wet weight), but β -LPH levels in the striatum (1 unit per g) are about 10-fold lower than in the hypothalamus (9–10 units per g) and are exceeded by levels in the central grey and thalamus (2.5 units per g).

We therefore reasoned that if the two systems are in fact separate, specific lesions may be able to differentiate between them. We produced unilateral lesions of the basal hypothalamus arranged so as to destroy the majority of the β -LPH-positive cells ($n=5$). The control group was sham operated ($n=5$). Ten days after surgery, the animals were killed, their brains rapidly removed and the cortex, septum, cerebellum and hippocampus dissected away. The remainder of the brain was divided into a posterior region containing the medulla-pons, central grey, tegmentum and colliculi, and an anterior region

Table 1 Effect of unilateral hypothalamic lesions on met-enkephalin and β -LPH in two regions

	Enkephalin (pmol per g tissue) Met-enkephalin	β -LPH: (units per g) β -LPH
Posterior region	Controls = 1,110 $\pm$ 193 Lesioned = 890 $\pm$ 230 %C = 80% Not significant	Controls = 5.0 $\pm$ 0.4 Lesioned = 2.3 $\pm$ 0.8 %C = 45% $P < 0.02$
Anterior region	Controls = 1,431 $\pm$ 85 Lesioned = 1,159 $\pm$ 165 %C = 81% Not significant	Controls = 2.5 $\pm$ 0.4 Lesioned = < 1.2 %C = < 50%

Lesions were placed in the basal hypothalamus unilaterally to destroy β -LPH cells. Brains were rapidly removed, dissected, frozen and homogenised with 0.4 M PCA. The supernatant contained met-enkephalin and the pellet β -LPH which were assayed by specific RIAs. The anterior region includes hypothalamus, thalamus and striatum. The posterior region includes central grey, medulla pons, tegmentum and colliculi. As lesions are unilateral, a 50% drop could indicate complete loss on one side (see text). A similar study of a lesion in the periaqueductal central grey produced major changes in met-enkephalin with minor changes in β -LPH levels. β -LPH values are expressed in units. This is necessary because the dilution curve of rat extracts is not parallel to human β -LPH standards. The quantitation is carried out in terms of a dilution curve of pituitary rat extract. At 50% inhibition, 1 unit corresponds to 1 pmol of human β -LPH.

involving thalamus, hypothalamus and striatum. The brains were rapidly frozen on dry ice, and β -LPH and enkephalin were extracted using 0.4 M perchloric acid as described elsewhere^{21,28}. Table 1 shows the results of β -LPH and enkephalin RIAs. As can be seen, β -LPH levels decreased significantly to about 50% of control, a maximal drop for a unilateral lesion. On the other hand, met-enkephalin levels showed a lower drop which did not reach significance in either brain part. It is important to note that such a differential effect cannot be attributed to differences in the turnover rates of the two substances, because in similar conditions lesions in the mesencephalic central grey produce the opposite pattern (that is, a significant decrease in enkephalin and no change in β -LPH levels in the anterior region)^{21,28}. The sites of lesions in these studies were immunohistologically confirmed in a separate group of animals.

As the above studies suggested the separability of β -LPH/ β -endorphin from the enkephalin systems, we proceeded to characterise the endorphin system further. Using the above antisera, we have studied the anatomical distribution of β -LPH, β -endorphin and ACTH in the same section of rat brain.

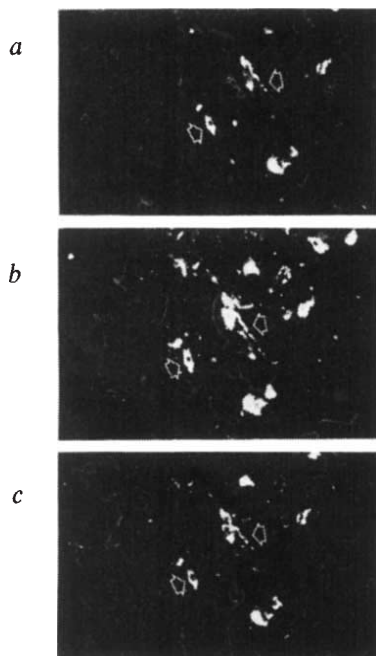
Male Sprague-Dawley rats were injected intracerebroventricularly with 50 μ g colchicine in 50 μ l of saline 48 h before death. Sections were prepared for immunocytochemistry as described elsewhere^{8,9}. Briefly, the rats were perfused with 4 g per 100 ml formaldehyde, their brains removed and blocked, re-exposed to formaldehyde solution followed by 10 g per 100 ml sucrose phosphate-buffered saline (PBS), frozen in liquid nitrogen and then sectioned in a -20°C cryostat. 10- μ m sections were incubated for 48 h with rabbit anti-ACTH antibody (1/1,500). They were washed and exposed to goat anti-rabbit-fluorescein isothiocyanate (FITC) tagged IgG (1/80), rewashed, viewed and photographed with a Leitz Orthoplan microscope. The sections then had their coverslips removed, were washed and exposed to 6 M guanidine HCl overnight (to break antigen-antibody bonds); their coverslips were then replaced, and they were viewed again. The areas photographed earlier were exposed to ultraviolet (UV) light until the residual fluorescence was completely exhausted, and then rephotographed. The section was washed again, and incubated with goat anti-rabbit IgG (without FITC) to block any remaining rabbit anti-ACTH antibody which had not been removed by guanidine HCl. The section was then exposed to the second antibody (β -endorphin) and the entire sequence repeated. The

third antibody (β -LPH) was visualised in the same way. Thus, exactly the same area could be studied for immunoreactivity to ACTH, β -endorphin and β -LPH.

All three antisera could be blocked by 1 μ M of the appropriate peptide. Several sections were run to demonstrate that goat anti-rabbit-FITC did not rebind to residual primary antibody, that guanidine HCl did not damage the immunoreactivity of these three peptides, that after guanidine HCl an antigen could be revisualised, and that guanidine treatment is not necessary for the demonstration of the second or third antigen. Further, some animals were run without colchicine to ascertain that this drug did not alter the pattern of immunoreactivities. These sections showed the same results, although cell body fluorescence was less intense.

Sections through the periaruate region of the hypothalamus were studied because cells for all three peptides (ACTH, β -LPH and β -endorphin) were found only in this area. Figure 1 shows the ACTH-positive (1a), β -endorphin-positive (1b) and β -LPH-positive (1c) cells in the arcuate region. All three antisera demonstrated the same cells and fibres. We observed no cells which contained one peptide and not the others. Fibres in the hypothalamus, medial amygdala and periventricular nucleus of the thalamus exhibited the same pattern for all three antisera. Fluorescence was absent in peptide-blocked (1 μ M) controls taken from serial sections (not shown). We have not yet seen any overlap between cells positive for the enkephalin system and those positive for ACTH/ β -LPH/ β -endorphin.

Fig. 1 Immunocytochemical demonstration of ACTH, β -endorphin and β -lipotropin immunoreactivity in the same hypothalamic neurones. Adult male Sprague-Dawley rats given 50 mg colchicine intracerebroventricularly, 48 h earlier; prepared for immunocytochemistry as in text. *a*, ACTH-positive cells in the periaruate region of hypothalamus (see arrows). Magnification $\times 200$. Fluorescence was completely lost after exposure to 6 M guanidine HCl and UV light. *b*, The same section, after exposure to 6 M guanidine HCl and UV light, was re-exposed to goat anti-rabbit IgG to block remaining rabbit IgG. It was then exposed to β -endorphin antiserum. The same cells are revisualised (see arrows). Magnification $\times 200$. Again, exposure to 6 M guanidine HCl and UV light eradicated fluorescence. *c*, Re-exposure to goat anti-rabbit IgG as in *b*, followed by incubation with β -LPH antiserum. The same cells and processes are seen in all three photographs. Excess peptide (1 μ M) blocked all fluorescence.



Using anatomical, biochemical and immunological tools, it has been possible to demonstrate the existence of two separate opiate peptide neuronal systems, one being an enkephalin system and the other a β -LPH/ β -endorphin/ACTH-positive system. During this work, Rossier *et al.*²⁹ reported that β -endorphin and enkephalin, as measured by RIA, are located in different proportions in several brain regions, again supporting the hypothesis of two separate opiate peptide systems. Although it is possible that β -endorphin serves as a precursor for met-enkephalin in enkephalin cells, it is also likely that it functions as a neuroregulator in its own neuronal pool. Further, it seems unlikely that met-enkephalin is purely a breakdown product of β -endorphin, as these peptides occur in morphologically separate systems and can be differentially altered by lesions. The relationships between these two neuronal-biochemical systems and between the peptides within each system remain to be determined.

We thank Dr C. H. Li for β -LPH and anti- β -LPH antiserum, Drs R. Mains and B. Eipper for anti-ACTH and anti- β -endorphin antisera and Ciba-Geigy for synthetic ACTH 1-24. This research was supported by NIMH Program-Project grant, MH 23861 and NIDA grants, DA 01207 and DA 01522. S.J.W. is supported by a Bank of America-Giannini Foundation Postdoctoral Fellowship and by an NIMH training fellowship. H. A. is supported by an Alfred P. Sloan Research Fellowship in Neurophysiology. J. D. B. holds NIMH Research Scientist Development Award, MH 24161.

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The role of GABA in the substantia nigra

STRIATONIGRAL GABAergic fibres are widely believed to exert a tonic inhibitory influence on the dopamine (DA)-containing cells of the substantia nigra (SN), and contraversive and ipsiversive circling behaviour can be explained in terms of a greater or lesser activity of the dopaminergic neurones, respectively^{1,2}. However, the blockade of γ -aminobutyric acid (GABA) receptors with intranigral picrotoxin occasionally stimulates rats to turn ipsiversively^{3,4}, rather than evoking the more commonly observed response in the opposite direction⁵. Similarly, injections of GABA agonists into the SN sometimes trigger an atypical contraversive turning syndrome^{3,4,6,7}. We describe here a study which sought to reconcile these paradoxical phenomena by investigating the effects on circling behaviour of decreasing the influence of GABA within the SN with picrotoxin, and of potentiating the action of GABA with *cis*-1,3-aminocyclohexane carboxylic acid (ACHC)⁸, a neuronal GABA uptake inhibitor. We show that the same drugs are capable of producing opposite turning responses when injected into the rostral and caudal parts of the SN. Moreover, behaviour patterns influenced by the anterior SN are accompanied by significant changes in homovanillic acid (HVA) levels in the striatum and require an intact DA system, whereas turning responses initiated by caudal drug injections are apparently not mediated by the dopaminergic nigrostriatal pathway.

Injections of picrotoxin (100 ng) into the rostral SN initiated immediate contraversive rotatory movements and a rise in striatal HVA concentration ipsilaterally, indicating that striatal DA utilisation had risen on the treated side of the brain (Fig. 1a and Table 1). By contrast, the administration of ACHC (10 ng) to the same area produced consistent ipsiversive circling and a drop in HVA formation in the corresponding striatum (that is, a fall in DA turnover). Control injections of physiological saline were never observed to alter the animals' behaviour or to modify striatal DA turnover. Groups of 12 rats receiving one or two doses of 6-hydroxydopamine (6-OHDA) showed, one week later, mean falls of 67.5% and 88.4%, respectively, in striatal DA levels. 6-OHDA treatment abolished the turning responses to both rostral picrotoxin and ACHC, and significant attenuation of both drug-induced behaviours was achieved with haloperidol (0.4 mg per kg, intraperitoneally) and electrocoagulation of the crus cerebri (Fig. 1a). From these data we conclude that the behavioural

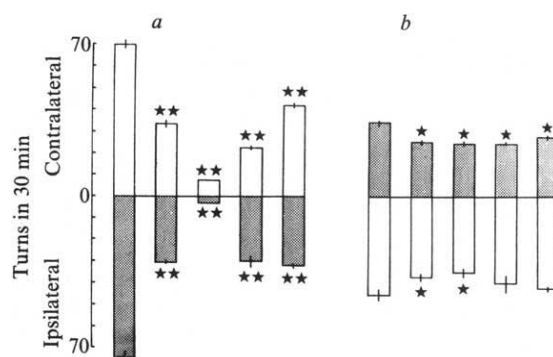


Fig. 1 Turning responses elicited by picrotoxin (open columns) and ACHC (shaded columns) injected into the rostral (a) and caudal (b) regions of the SN (see legend to Table 1). Results are shown for injections made into untreated control animals (first columns, $n = 12$), for groups of six rats pretreated with one (second columns) or two doses (third columns) of 6-hydroxydopamine unilaterally into the median forebrain bundle (4 μ g in 2 μ l, one week earlier), or haloperidol (0.4 mg per kg, intraperitoneally, 30 min previously) (fourth columns), and for rats with one-week-old unilateral electrolytic lesions of the crus cerebri (fifth columns, $n = 6$). Electrolytic lesions were made by implanting fine concentric bipolar electrodes into the crus cerebri close to the mammillary bodies and passing a direct current of 1 mA for 10 s. $\star P < 0.01$, $\star\star P < 0.001$ compared to controls (Student's *t*-test).

responses to picrotoxin and ACHC, delivered discretely into the anterior region of the SN, were caused by modifying the levels of endogenously released GABA and thereby altering the activity of the DA cells.

We found it was also possible to make rats rotate by injecting either of these two drugs into the caudal area of the SN. Surprisingly, the direction of turning was now opposite to that evoked by rostral drug placements (Fig. 1b). We have no evidence that these caudally evoked responses involved the nigrostriatal DA system, as there were no corresponding changes in striatal HVA production (Table 1) and the behaviours were scarcely attenuated by successful lesions of the median forebrain bundle (Fig. 1b). Similarly, haloperidol only slightly reduced ACHC-induced contralateral rotation, whereas picrotoxin-induced ipsilateral movements were completely insensitive to DA receptor blockade. Interestingly, both drugs were more or less equally effective when administered to the caudal SN of crus cerebri-lesioned rats, suggesting to us that the GABA afferents to the anterior and posterior areas of this nucleus may follow separate paths.

The finding that the direction of turning elicited by intranigral injections of picrotoxin and GABA-like drugs depends on whether the compounds are delivered rostrally or caudally in the SN offers a simple explanation for the contrasting behavioural actions of these drugs that have been reported by different investigators^{4,7,12}. The traditional notion of an inhibitory link between GABA- and DA-containing neurones in the rostral SN therefore seems sound, but it does not account for the opposite action of GABA in the caudal SN. Two possible explanations warrant consideration. Where contraversive rotation induced by the intranigral administration of GABA-like drugs¹² can be related to increased nigrostriatal dopaminergic activity¹³, it may be conjectured that the compounds are acting indirectly by suppressing the activity of nigral inhibitory interneurons, thereby de-inhibiting (that is, exciting) the nigrostriatal DA pathway^{13,14}. On the other hand, we have found that behavioural responses elicited by modifications to the GABA system in the caudal SN are resistant to inhibition by procedures which attenuate nigrostriatal DA cell activity. We interpret this to mean that a second

Table 1 Influence of intranigral picrotoxin and ACHC on striatal HVA concentration

Drug treatment	Placement	Striatal HVA concentration (μ g per g wet weight)		<i>P</i>
		Contralateral	Ipsilateral	
Picrotoxin	Rostral	0.35 $\pm$ 0.02	0.69 $\pm$ 0.04	< 0.001
	Caudal	0.38 $\pm$ 0.02	0.36 $\pm$ 0.02	NS
ACHC	Rostral	0.39 $\pm$ 0.02	0.23 $\pm$ 0.02	< 0.001
	Caudal	0.41 $\pm$ 0.02	0.46 $\pm$ 0.02	NS

Male Wistar albino rats (Tuck, 190–210 g) were anaesthetised with halothane and injected stereotactically (0.1 μ l over 5 min) with picrotoxin or ACHC into the rostral (A2.5, L2, D-2) or caudal (A1.6, L2, D-2) regions of the SN⁹. On recovery, rats were placed singly in cages and the numbers of complete revolutions made in a minute were scored every 5 min for a total of 30 min. DA¹⁰ and HVA¹¹ were assayed fluorimetrically in corpora striata dissected from the brains of separate groups of rats injected intranigral with the drugs and killed after 15 min when the frequency of turning was highest. Each value is the mean $\pm$ s.e.m. of six separate experiments. NS, not significant by Student's *t*-test.

system of non-dopaminergic (excitatory?) neurones originates in the region of the SN and cooperates in the control of motor activity and that these, too, are under inhibitory GABA control.

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Received 16 March; accepted 26 June 1978.

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Striatal dopamine release and contraversive rotation elicited by intranigraly applied muscimol

SEVERAL lines of evidence indicate that release of dopamine (DA) within the corpus striatum mediates turning behaviour in the rat^{1,2}. The turning occurs in a direction away from the striatum with the greatest activation of DA neurones. Contraversive turning also occurs after unilateral microinjection of agonists of γ -aminobutyric acid (GABA) into the substantia nigra (SN)^{3–6}, the site of origin of the dopaminergic nigrostriatal pathway. This finding was unexpected, as GABA inhibits neurones^{7,8} thought to be DA-containing cells^{9,10} in the SN. Hence, a GABA agonist would be expected to cause a decrease in the rate of release of striatal DA on the microinjected side and result in ipsiversive turning. Therefore, it is unclear whether a GABA agonist applied to the SN alters DA release in the ipsilateral striatum to evoke turning, or whether the turning results from a non-dopaminergic mechanism⁵. The dopamine antagonist, haloperidol, has been reported either to reduce significantly^{3,6} or exert no action on^{4,5} the contraversive turning response evoked by intranigral injection of a GABA-like compound. To clarify the action of GABA on the nigrostriatal dopaminergic system, we examined the effect of muscimol, a potent GABA agonist¹¹, on striatal DA metabolism and release. We report here that intraperitoneally (i.p.) administered muscimol increases the concentration of striatal homovanillic acid (HVA) and that intranigral perfusion of muscimol simultaneously evokes both contraversive turning and DA release from the ipsilateral striatum.

The effect of peripherally administered muscimol on the striatal levels of DA and its major metabolite, HVA, are given in Table 1. The concentration of striatal HVA was increased significantly at 0.5, 1 and 2 h after muscimol (2 mg per kg, i.p.) injection, whereas the concentration of DA was not altered. These results agree with those obtained in mice¹² and suggest that systemically administered muscimol can stimulate turnover or release of DA in the striatum.

Table 1 Effect of muscimol on rat striatal dopamine and homovanillic acid

Time (h) after muscimol	Concentration (nmol per g+s.d.)	
	Dopamine	Homovanillic acid
0	21.5 ± 3.3	1.9 ± 0.5
0.5	21.2 ± 7.8	2.8 ± 0.6*
1.0	24.3 ± 6.3	3.5 ± 1.1*
2.0	22.2 ± 7.9	4.0 ± 1.6*
4.0	20.9 ± 4.9	2.3 ± 0.9

Male Sprague-Dawley rats (Charles River, $n = 6$ per group) were given muscimol (2 mg per kg) intraperitoneally. Zero time control rats were treated with the vehicle (0.9% saline). Dopamine and homovanillic acid were extracted simultaneously from the same tissue sample and measured by a fluorometric method as described previously²⁵.

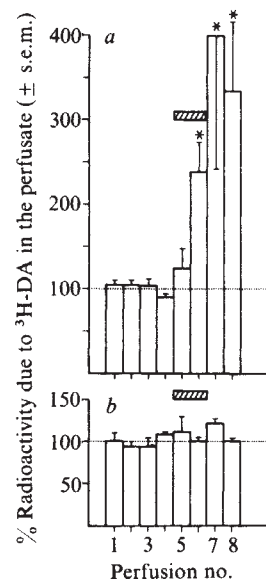
* Different from vehicle-treated control, $P < 0.02$ (Student's t test).

To evaluate the effect of muscimol on DA neurones further, the striatal release of the neurotransmitter was studied, using a push-pull cannula, while simultaneously perfusing a solution of muscimol through a second push-pull cannula in the ipsilateral SN. For push-pull perfusion, seven male Sprague-Dawley rats weighing 480–550 g, with a guide cannula chronically implanted in the corpus striatum and SN, were used. One week after surgery, a concentric push-pull cannula¹³ was lowered into the striatum and an artificial cerebrospinal fluid (CSF)¹⁴ containing 50 μ Ci ml⁻¹ of ³H-tyrosine (40–50 μ Ci mmol⁻¹) was perfused at a rate of 30 μ l min⁻¹. The experiment, modified from that of Nieoullon *et al.*¹⁵, consisted of eight serial 15-min perfusions of the striatum. During the fifth and sixth striatal perfusion, 10⁻⁴ M muscimol or the CSF vehicle was perfused simultaneously through the SN. A 400- μ l aliquot of each striatal perfusate was assayed for ³H-DA content using the ion-exchange method of Neff *et al.*¹⁶.

Each animal was used in two experiments spaced by a one-week interval. CSF was perfused through the SN in one and muscimol (10⁻⁴ M) in the other. Muscimol was used first in four

Fig. 1 The mean ($\pm$ s.e.m.) ³H-DA efflux detected in eight consecutive push-pull perfusates collected in the striatum. The radioactivity due to ³H-DA is expressed as a % of the spontaneous release value (indicated by the dotted line) as defined in the text. The horizontal bars (▨) indicate the periods during which either muscimol (10⁻⁴ M) (a) or CSF (b) was simultaneously perfused through the SN.

* Significant difference between groups ($n = 5$) using the Mann-Whitney U -test ($P < 0.002$).



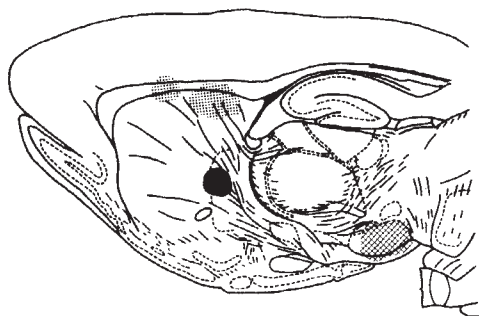


Fig. 2 A sagittal section of the rat brain depicting the areas perfused. The stippled section in the dorsal striatum encompasses the sites at which ^3H -tyrosine was perfused and ^3H -DA release was measured ($n=6$). The cross-hatched section in the SN encompasses the area in which muscimol was perfused ($n=7$), whereas the site in the globus pallidus at which no increased ^3H -DA release was noted during the muscimol perfusion is indicated by the black spot.

animals. The mean amount of ^3H -DA in the four perfusates preceding the SN perfusion was used as a value indicative of spontaneous ^3H -DA release (SR). ^3H -DA in each perfusate was expressed as a percentage of the SR and compared between muscimol and CSF perfusions using the Mann-Whitney *U*-test. The mean SR for 56 perfusates was 3.35 ± 0.62 (s.e.m.) nCi per ml of effluent. Each perfusion site was verified using the atlas of König and Klippel¹⁷.

In five rats, both contraversive turning and a significant efflux of ^3H -DA within the ipsilateral striatum were observed. As shown in Fig. 1, the efflux of ^3H -DA rose significantly during the muscimol perfusion and remained elevated in the subsequent perfusates. ^3H -DA release reached a maximum of 400% of SR in the first striatal effluent collected after stopping muscimol perfusion. The contraversive turning response to muscimol began with latencies varying between 30 s and 8 min, possibly due to slight differences in cannula placement. As with the release of ^3H -DA, contraversive turning and postural asymmetries also persisted after the cessation of the muscimol perfusions. Perhaps the persistent ^3H -DA efflux, circling behaviour, and contralateral postural asymmetries were caused by muscimol which had diffused into surrounding tissue. If as little as 0.01% of the perfused muscimol solution had diffused into the SN, it would be equivalent to a 1-ng microinjection of muscimol, a dose which has been reported to evoke contraversive turning⁵.

Increase of ^3H -DA release occurred only during or following the perfusion of muscimol which concomitantly evoked contralateral turning. Hence, only animals which both turned contraversively in response to muscimol and had verified neo-striatal and nigral perfusion sites were included in the group data. In one animal which did not turn during the muscimol perfusion, there was no increase in ^3H -DA in the striatal perfusate. As shown in Fig. 2, one striatal perfusion site was actually in the globus pallidus. Although this animal turned during the muscimol perfusion, no significant ^3H -DA efflux was observed from the pallidal site.

The SN perfusion of the potent GABA agonist, muscimol, both releases ^3H -DA in the ipsilateral striatum and evokes contraversive turning, supporting the hypothesis that muscimol-evoked turning and striatal DA release are causally related and suggesting that striatal DA mediates the turning. Muscimol may act to release DA neurones from tonic inhibition mediated by other SN neurotransmitters which include: substance P (ref. 18), acetylcholine¹⁹, serotonin¹⁹, dendritic DA²⁰ and glycine²¹. Using the 'encephalé isolé' preparation in the cat, Chéramy *et al.*²²⁻²⁴ have already demonstrated that several of these putative transmitters can modulate striatal ^3H -DA release when they are perfused through the SN. Future studies of the effects of

muscimol on these substances may help to determine the precise mechanism whereby intranigally administered muscimol evokes both contraversive turning and striatal DA release.

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Received 30 May; accepted 4 August 1978.

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Coupled motoneurones are part of the crayfish swimmeret central oscillator

SWIMMERETS in the crayfish are the paired ventral abdominal appendages which beat in a metachronal rhythm during behaviours such as swimming and burrow ventilation. Each swimmeret is driven by alternating bursts of impulses in antagonistic power- and return-stroke motoneurones. Although proprioceptive input affects the detailed structure of the rhythm, the basic motor programme can be elicited from the deafferented abdominal central nervous system (CNS) by tonic stimulation of single 'command fibres' in the ventral nerve cord¹⁻³. Each swimmeret is controlled by a hemiganglion capable of generating the rhythm in isolation, although normally the most posterior swimmeret ganglion (the 5th) acts as a pacemaker, regulating the activity of the other ganglia through a series of coordinating fibres located in the medial region of the ventral nerve cord⁴. I report here the preliminary results of an investigation into the structure of the central neural oscillator controlling swimmeret movements in crayfish. The results suggest that motoneurones in this system do not act merely as output elements which passively relay the activity of an oscillator formed from pre-motor neurones within the CNS, but that the motoneurones themselves are an integral part of the pattern generating circuits. There is both excitatory and inhibitory central coupling between motoneurones mediated either by electrical or graded chemical synapses. Furthermore, the activity of some motoneurones can directly influence the intensity, period, and phase of the oscillation.

Experiments were performed on the 4th abdominal ganglion of *Pacifastacus leniusculus*, which was attached to the rest of the

abdominal CNS, but isolated from the peripheral and rostral nervous system. Intracellular recordings were made with microelectrodes penetrating neuropilar regions of motoneurons from the dorsal surface of the desheathed ganglion. Extracellular pin electrodes were placed on the anterior and posterior branches of the 1st root, for recording and stimulating return-stroke and power-stroke motoneurons respectively. In some of the experiments reported here rhythmic motor output could be elicited by stimulating either the anterior or posterior branch of the 1st root at 25 Hz for 1–2 s. No attempt was made to stimulate command fibres. Occasionally, spontaneous rhythmic output was observed without any prior stimulation. The results of one typical experiment are described in detail below and then a summary is given of conclusions derived from a number of experiments.

A neurone was identified as a power-stroke motoneurone because it had an axon in the posterior branch of the 1st root, but not in the anterior branch (Fig. 1*a, b*). During spontaneous rhythmic output its membrane potential showed subthreshold oscillations, receiving excitatory postsynaptic potentials (e.p.s.ps) late in the burst of spikes recorded from other neurones in the posterior 1st root, and inhibitory post-synaptic potentials (i.p.s.ps) during the burst in the anterior 1st root (Fig. 1*c*). It was thus likely to be a fast excitor power-stroke motoneurone⁵. Injection of subthreshold depolarising current into this neurone when the rhythmic output was not being expressed caused an increase in the tonic spike frequency of other neurones with axons in the posterior 1st root. Conversely, hyperpolarising this neurone caused a decrease in the spontaneous spike frequency of such neurones (Fig. 2*a*). Since these effects result from subthreshold changes in membrane potential in a CNS isolated from the periphery, I conclude that this power-stroke excitor motoneurone is coupled to other power-stroke neurones either through nonrectifying electrical synapses or through graded chemical synapses in which there is a continuous release of excitatory transmitter by the resting neurone which can be increased by depolarisation and decreased by hyperpolarisation⁶.

This motoneurone was also coupled to pre-motor elements. When the motoneurone did not receive rhythmic input, it often received a tonic barrage of apparently unitary e.p.s.ps at ~20 Hz, which was continuous for many minutes with a very stable frequency. The frequency of this barrage was not affected by low levels of injected current (which were sufficient to

Fig. 1 Identification of a fast power-stroke excitor motoneurone. *a*, Triggering the oscilloscope sweep from a spike recorded intracellularly (lower trace, induced by injecting depolarising current) shows a phase-locked spike recorded extracellularly in the posterior (power-stroke) branch of the 1st root (middle trace) but none in the anterior (return-stroke) branch (upper trace). *b*, Stimulating the posterior branch causes an antidromic spike (arrow) which is recorded intracellularly. A spike is also recorded in the anterior branch, but not consistently. *c*, During periods of oscillator activity, the motoneurone (upper trace) receives rhythmic subthreshold depolarisations approximately in phase with bursts of spikes of power-stroke motoneurons in the posterior branch (lower trace) and in antiphase with the weak burst of spikes of return-stroke motoneurons in the anterior branch (middle trace).

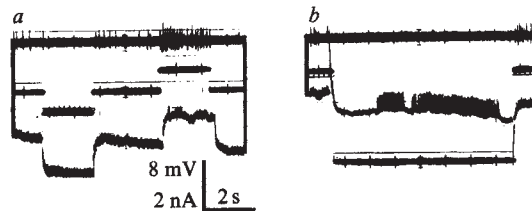
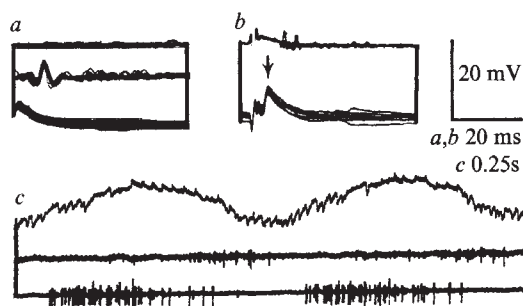


Fig. 2 *a*, Hyperpolarising current (middle trace) injected into the motoneurone (lower trace, bridge circuit only approximately balanced) decreases the spontaneous spike frequency of units in the posterior branch (upper line), while depolarising current increases their frequency. *b*, Hyperpolarising the motoneurone partially inhibits an excitatory presynaptic neurone.

demonstrate the motoneurone coupling), but with larger amplitudes of hyperpolarising current, the e.p.s.p. barrage became discontinuous (Fig. 2*b*). When present, the e.p.s.ps still occurred at ~20 Hz, but the previously continuous barrage was now broken by silent periods up to several seconds in duration. The e.p.s.ps were augmented in amplitude by the hyperpolarisation and thus were probably chemical. No spikes recorded in the anterior or posterior 1st root were phase locked to these e.p.s.ps, and so they are unlikely to be the result of reciprocal coupling from another motoneurone. Whatever the origin of these e.p.s.ps, the result demonstrates that this power-stroke excitor motoneurone is coupled to its own pre-motor excitor neurones, which suggests a positive feedback loop within the CNS.

When the CNS was producing the rhythm which in the intact animal causes swimmeret beating, the parameters of the oscillation could be altered by injecting current into this single motoneurone. Hyperpolarising current (~3 nA) caused a reduction in the number of units active during the power-stroke phase of the cycle and an increase in the activity of units in the return-stroke phase of the cycle. There was also a decrease in the frequency of oscillation and a disturbance of the strict reciprocity of power- and return-stroke activity (Fig. 3*a*). This could be seen not only in terms of the motor output as recorded extracellularly in the 1st root, but also as the synaptic input impinging upon the motoneurone. The i.p.s.ps during the return phase disappeared, probably because the membrane potential was near their reversal potential, while the e.p.s.p. barrage of the power-stroke phase became prolonged. These e.p.s.ps were similar in shape and intra-burst frequency to those described above in the quiescent preparation, but their identity remains a matter of speculation. More extreme hyperpolarisations (~5 nA) eliminated the rhythmic motor output altogether. Injection of small depolarising currents (1–2 nA) increased the number of units active in the power-stroke phase (including recruiting spikes in the injected motoneurone itself) and decreased the activity during the return-stroke phase. Hyperpolarising currents had the opposite effect (Fig. 3*b*). The depolarising current had no significant effect on the period of the rhythm, but the hyperpolarising current slowed it by 6% ($P < 0.025$). Continuous injection of larger depolarising currents strongly inhibited return-stroke activity, and increased the activity of a number of power-stroke neurones to the extent that it became difficult to distinguish any rhythmicity in the motor output. However, injection of pulses of such depolarising current had a strong resetting effect on the oscillator, including both the power- and return-stroke phases of the rhythm (Fig. 3*c*).

The levels of current used in these experiments were low (≤ 5 nA), and thus it is likely that the changes in motoneurone membrane potential occurring in the absence of experimental interference are effective in producing the central interactions. Much larger currents (10–20 nA) injected into extracellular space immediately after withdrawing the microelectrode from the neurone had no effect. The preparation remained healthy for several hours, and both the coupling to other motoneurons and the effects on the oscillation of injecting current into the

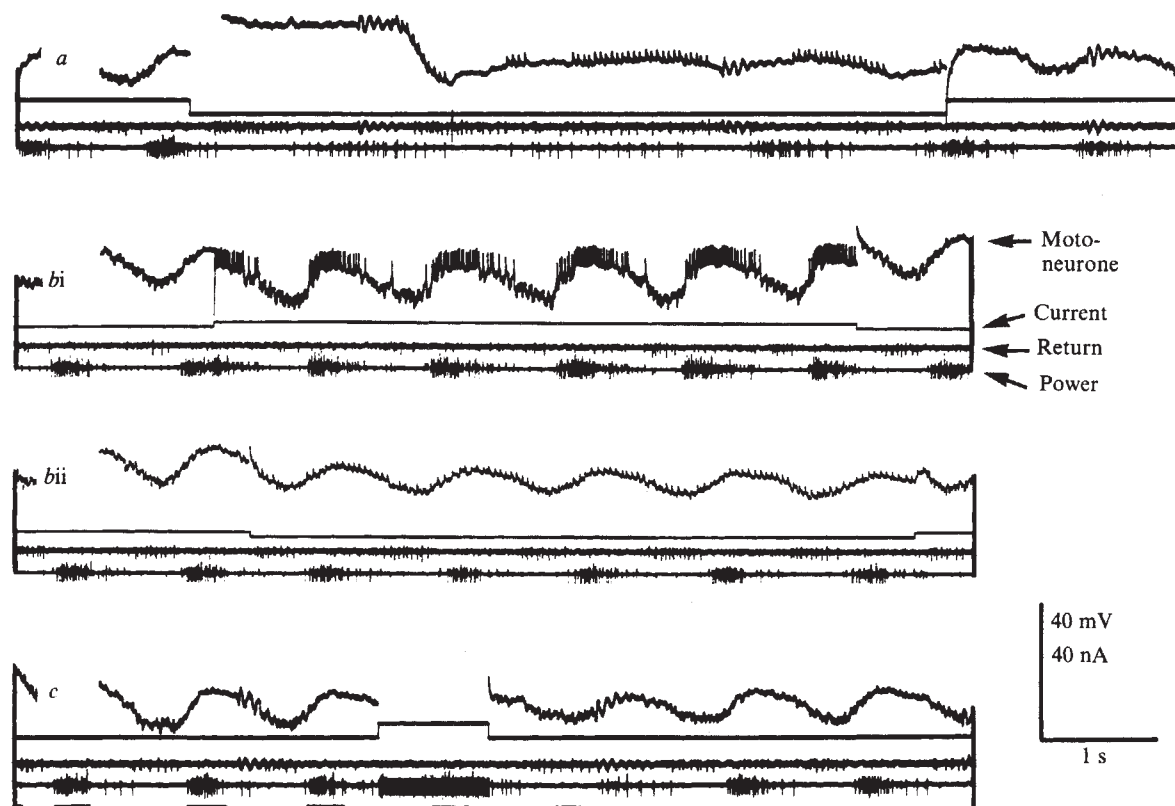


Fig. 3 *a*, Hyperpolarising current (2nd trace) injected into the motoneurone (upper trace) reduces the intensity of the power-stroke motor activity (bottom trace) and increases the period of the oscillation. There is greater than usual overlap between power- and return-stroke (3rd trace) activity. (The apparent motoneurone hyperpolarisation about 2 s after the start of current injection is caused by a change in the bridge balance.) *b*, Small depolarising currents (i) increase the intensity of power-stroke activity and decrease the intensity of return-stroke activity, while small hyperpolarising currents (ii) have the opposite effect. Note the slight difference in period between (i) and (ii). *c*, Injecting a pulse of depolarising current (bridge unbalanced) can reset the phase of the oscillator. The solid bars under the record indicate the times at which power-stroke activity would have been expected had there been no resetting.

motoneurone could be demonstrated repeatedly and consistently.

The oscillator has been reset by injecting current (<5 nA) into a single neurone in a total of four separate preparations—twice when depolarising current was injected into power-stroke motoneurons, and twice when depolarising current was injected into neurones which did not spike in response to depolarising current (up to 10 nA). Two other power-stroke motoneurons did not reset the oscillator when injected with similar levels of depolarising current. This variability may be due to differences in the sites of current injection relative to central output synapses of the motoneurons. Alternatively it may reflect genuine differences within the population of motoneurons. This latter is quite likely, since the lobster swimmeret motor pool consists of about 50 motoneurons innervating 11 muscles per swimmeret⁵, and the crayfish swimmeret system is probably just as complex. The gross distinction of power- and return-stroke motoneurons used in this work takes no account of basipodite rotators, exopodite openers, closers, benders and so on which cannot yet be identified in the isolated central nervous system. To determine what proportion of the total motor pool has access to the oscillator will require many more experiments.

Penetrations of power- and return-stroke motoneurons in more than 10 preparations which were not oscillating (and whose motoneurons could therefore not be tested for access to the oscillator) has revealed that coupling between motoneurons is widespread. Only rarely have motoneurons been found which did not exhibit some form of coupling. In general, this coupling is organised such that depolarising current

injected into one motoneurone excites its synergists and inhibits its antagonists, while hyperpolarising current has the opposite effect. In at least two pairs of power-stroke motoneurons the coupling is reciprocal and seems to be the result of an electrical synapse. Interactions are strongest between ipsilateral motoneurons within a ganglion, but have also been shown between motoneurons in adjacent ganglia. As yet no coupling has been found between contralateral motoneurons.

These results show that in the neural system controlling swimmeret movements the activity of at least some motoneurons can influence the intensity, frequency, and phasing of the rhythmic motor output from an isolated CNS. This suggests that these motoneurons themselves are part of, or at least can interact with, the oscillator circuit, and are thus not simply passive output elements. Furthermore, since none of the motoneurons from which I have recorded are endogenous pacemakers, the parameters of the oscillator must be a property of a network of neurones. The coupling between motoneurons demonstrated in the quiescent CNS may be a result of direct connections between the motoneurons, or may be mediated by interneurons, perhaps some of the same interneurons as those involved in the oscillator circuit.

This work was supported by USPHS grant NS 12295 and the Alfred P. Sloan Foundation. I thank Professor B. Mulloney and Ms G. Jacobs for help and encouragement.

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Received 8 May; accepted 10 July 1978.

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Evidence for the functional involvement of carotenoids in the photoperiodic reaction of spider mites

PHOTOPERIODISM in insects and mites depends on the presence of light-absorbing pigments, which are probably located in the central nervous system; photoperiodic responses are mediated by the direct action of light on these nervous elements¹. Until now, pigments involved in photoperiodic processes such as diapause and polymorphism have not been identified. Different groups of pigments have been named by various authors as possible candidates for this function. A biologically active role based mainly on action spectra studies and the absorption characteristics of isolated pigments has been suggested for pterins², carotenoids³, a combination of carotenoproteins, flavoproteins and haemoproteins⁴ and bile pigments⁵. Carotenoids, pterins and flavins have also been proposed as possible photoreceptor pigments in studies on the spectral sensitivity of circadian rhythms⁶. According to Lees¹, however, the usefulness of action spectra in identifying the light acceptor is limited, as spectra for photoperiodic action cannot at present be matched against absorption curves. A different approach was used by Zimmerman and Goldsmith⁷, who reared *Drosophila* on a carotenoid-free diet and showed that in one generation the sensitivity of the visual receptors was lowered by 3 log units because of lack of rhodopsin. At the same time the photosensitivity of the rhythm of pupal eclosion in the cultures was not affected, from which it was concluded that the pigment involved in mediating light effects on the circadian rhythm in *Drosophila* is not a carotenoid derivative. We demonstrate here that carotenoids are functionally involved in the photoperiodic induction of diapause in the spider mite *Tetranychus urticae* using a method involving pigment mutants.

In *T. urticae*, only adult females may undergo a facultative diapause, induced by short-day photoperiods experienced during the larval and nymphal stages. With the test regime used (10L:14D, 18 °C) the different wild-type strains showed a 100% diapause⁸. From a wild-type strain we have isolated seven different pigment mutants, all due to single recessive mutations which interfere with the uptake and oxidative metabolism of β -carotene in these mites⁹. It seemed that only the albino mutant showed a lowered diapause response, all other pigment mutants exhibiting 100% diapause in the above test regime. Four albino mutants, which arose spontaneously in different wild-type strains, all responded with a lowered diapause, although all four originated from wild-type strains which give 100% diapause in the test conditions. Complete diapause has never been observed in any albino strain; although highly variable in expression (diapause responses range over 0-80% in different tests), the percentage diapause in albino strains is always markedly reduced in comparison with wild type. Back-crossing of three of the albino mutants to wild type for 10 generations did not restore the capability to diapause. As crossing-over is thought to occur very frequently in these mites¹⁰, these findings are a strong indication for a functional involvement of the albino locus in the photoperiodic reaction of *T. urticae*.

Even stronger evidence for a functional connection between the presence of carotenoid pigments and diapause was obtained in other crossing experiments between wild type and albino. For these crosses use was made of the male haploidy (arrhenotoky)

in *T. urticae*. The out-cross of the albino mutant to wild type gives hybrid daughters which are phenotypically wild type and diapause normally. Crossing these hybrid females to albino males produces a mixed female offspring, consisting of wild type and albino in a 1:1 ratio. Surprisingly, both these categories show a 100% diapause when tested in the photoperiodic regime mentioned above. Albino females of this offspring, after random mating with their brothers, which also consisted of a 1:1 ratio of wild type and albino, again gave rise to a mixed offspring of wild-type (hybrid) and albino females. From these, the wild-type females all diapaused on short-day treatment, whereas the albino daughters showed the same lowered diapause response as described for the different albino strains. From these crosses it seems that 100% diapause in albino is realised only when the albino daughters come from hybrid, phenotypically wild-type mothers. Apparently a maternal effect is responsible for the complete induction of diapause in albino females.

The lowered diapause response encountered in all albino strains and the fact that the capability to diapause could not be restored by a series of back-crosses to wild type, suggest that the albino locus exerts a pleiotropic effect, influencing both pigmentation and diapause induction. The discovery of the maternal effect in the crossing experiments described above confirms this supposition and leads us to conclude that carotenoids are functionally involved in diapause induction in *T. urticae*. With respect to pigmentation, a maternal effect, consisting of small red eyespots in albino males produced by wild-type hybrid females, has been demonstrated previously in these mites¹¹. This showed that the egg of spider mites transmits a certain amount of carotenoids, which may be used for the formation of eyespots. From the experiments described above it could be concluded that these maternally derived carotenoids may also be utilised by the embryo in the formation of a photopigment concerned in the induction of diapause. Very small amounts of pigment would probably suffice for this function. A more elaborate account of these and other results will be published elsewhere.

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Received 15 June; accepted 28 July 1978.

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Evidence for passive electrotonic interactions in red rods of toad retina

SINCE Baylor, Fuortes and O'Bryan¹ first demonstrated interactions between vertebrate photoreceptor cells, many types of inter-receptor influences have been described²⁻¹⁰. While some of these interactions are mediated by horizontal cells^{1,2,6}, others apparently result from direct contact between the photoreceptors themselves^{1,3,4,7-10}. Fain *et al.*¹⁰ reported that the red rods of the toad *Bufo marinus* were coupled by gap (electronic) junctions, and that single rods received detectable signals from other receptors over a retinal area of about 0.5 mm². This

receptive field area is 4–100 times larger than those measured for photoreceptors of a variety of species^{1,4,7,9,11,12,15}. Fain *et al.* found this extensive spread of rod signals to be inconsistent with network models containing only passive elements and this raised the possibility that some active process, perhaps in the rod membrane, contributed to the spread of rod signals. We report here results which indicate that single rods sum signals over only 1/16th the area previously estimated; this smaller receptive field area implies more intrinsic noise, a larger response to single photon absorptions, and a larger signal-to-noise ratio than predicted by the previously reported results¹⁰. Furthermore we find that, as in other species (ref. 13, and Copenhagen and Owen, personal communication), the spread of low-amplitude, light-evoked responses between photoreceptors can be accounted for by purely passive electrical properties.

Bufo marinus red rods were impaled in eye cup preparations and identified by their characteristic slow waveforms^{3,14,15}, spectral sensitivities^{14–17}, and by injection of Procion yellow. Receptive field properties were determined using two different stimulus configurations.

Two of us (H.F.L. and D.R.C.) recorded rod responses to a long, narrow bar of light flashed at various regions of each rod's receptive field. This bar was moved across the retina in a stepwise fashion, perpendicular to the long axis of the stimulus, and at positions separated by either 9.0 or 13.5 μm the stimulus was flashed from three to six times. Multiple responses, recorded to flashes at each position, were computer averaged and the peak amplitudes plotted semi-logarithmically as a function of slit displacement (Fig. 1). For all cells tested, the light response decreased exponentially as the bar was moved from the centre to the periphery of the receptive field. Such behaviour is predicted by two-dimensional cable models and permits the spatial properties of the response to be characterised by a length constant λ (refs 18, 19). For 11 rods tested, the mean length constant was $24 \pm 5 \mu\text{m}$ ($\pm$ s.d.) (Table 1).

In independent experiments, receptive field properties of toad rods were measured by one of us (R.A.N.) using flashed circular spots of light of increasing diameter. A small, dim spot of light (13–30 μm diameter) was centred over the most sensitive region of the rod's receptive field, and photoresponses were recorded as the diameter of the spot was increased from 13 to 265 μm at a constant, dim light intensity (Fig. 2, inset). For the cell shown in Fig. 2, the response to these flashes increased asymptotically to a diameter exceeding 200 μm . The diameter of the summation area (95% V_{FF}) was taken as 160 μm . The continuous curve represents the relationship between spot diameter and central voltage response for a continuous two-dimensional resistive sheet with a length constant of 18 μm . This relationship is described by

$$V(d) = V_{\text{FF}} \left\{ 1 - \left[\frac{d}{2\lambda} K_1 \left(\frac{d}{2\lambda} \right) \right] \right\}$$

where $V(d)$ is the voltage at the centre of the illuminated circle of diameter d , V_{FF} is the voltage with full field illumination, and K_1 is a modified Bessel function^{18,19}. For 14 rods tested in this manner, the mean length constant was $21 \pm 6 \mu\text{m}$ ($\pm$ s.d.).

These results indicate that red rods are functionally coupled over an area of approximately 0.03 mm^2 rather than 0.5 mm^2 as previously estimated¹⁰. Thus, each rod is functionally coupled to a maximum of 500–550 other rods rather than to 8,000–9,000 (refs 10, 20). We have also learned that two other investigators (ref. 21, and G. Gold, personal communication), recording from red rods in isolated *Bufo* retinal preparations (rather than in eye cups), have found summation areas comparable to those reported here.

This smaller receptive field size has several implications for the physiology of *Bufo* red rods. For example, the magnitude (r.m.s. value) of the intrinsic noise (membrane events not caused by the absorption of a photon) in a coupled rod should be inversely related to the diameter of the summation area (proportional to λ); this assumes that coupling serves to average

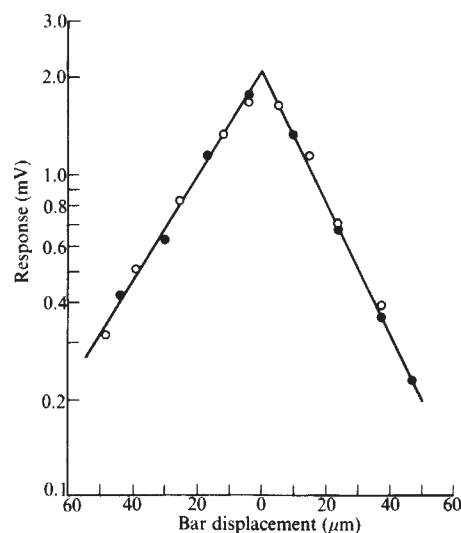


Fig. 1 Response of a *Bufo marinus* red rod as a function of the displacement of a narrow stimulus bar. The bar was moved through the receptive field of each cell twice. ○, Data collected during the first pass of the bar; ●, data collected as the bar was moved in the opposite direction. Points are the computer average of three to six responses at each position. Solid lines were fitted by the method of least-squares error. The bar was produced by passing the light beam through a rectangular slit formed by two razor blades. The image of the slit on the retina had a nominal halfband width of 8 μm as determined by passing the image across a 5- μm entrance aperture to a photomultiplier tube which was in the position normally occupied by the eye cup. The length of the bar was limited by an iris diaphragm and was 750 μm in the central position. Responses were elicited by 40-ms flashes of 514 nm light delivering to the retina about 120 photons per μm^2 per flash. Details of the optical system and its calibration are described elsewhere⁷.

noise sources across many rods as it does with turtle cones¹³. Thus, the smaller summation area would increase estimates of intrinsic noise by a factor of ~ 4 . Similarly, estimates of the coupling resistance between *Bufo* red rods would be larger for this smaller summation area, and one would expect less shunting of signals to adjacent rods. Quantitatively, estimates of input resistance, and hence the signal generated by the absorption of a single photon, would be increased by a factor of ~ 10 . Therefore,

Table 1 Summary of length constants for 11 *Bufo marinus* red rods

Rod no.	λ_-	r_-^2	λ_+	r_+^2	V_{centre} (mV)
1	25.9	0.99	24.6	0.97	2.2
2	26.1	0.96	24.6	0.97	3.4
3	33.8	0.99	18.9	0.98	2.7
4	24.2	0.98	23.2	0.98	2.2
5	22.3	0.94	17.8	0.98	2.0
6	24.0	0.99	25.4	0.97	2.4
7	21.7	0.98	24.7	0.96	1.0
8	31.3	0.99	28.8	0.95	2.3
9	26.3	0.99	21.7	0.99	2.7
10	28.3	0.99	25.0	0.97	2.2
11	15.5	0.99	12.6	0.99	1.8

λ_- and λ_+ represent length constants on opposite sides of the centre for individual rods; r_-^2 and r_+^2 are the coefficients of correlation between the data and a simple exponential function (Fig. 1). V_{centre} is the amplitude of the light response when the stimulus bar was over the central (most sensitive) part of the field. Differences between λ_- and λ_+ could not be attributed to retinal position or optical factors. Similar asymmetries have been seen in other species (ref. 18, and Copenhagen and Owen personal communication). The mean of these 22 length constants is $23.9 \pm 4.7 \mu\text{m}$ ($\pm$ s.d.).

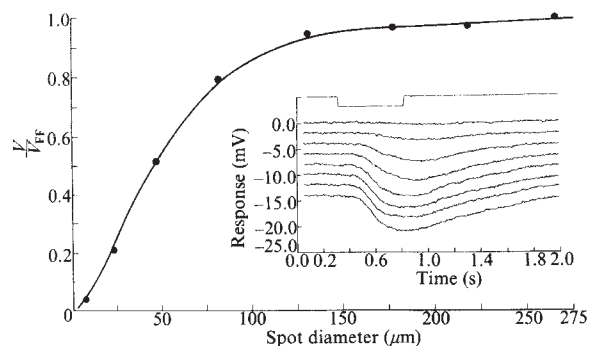


Fig. 2 Amplitude of photoresponses (circles) to 500-ms flashes of a dim circular spot of white light as its diameter was increased from 13 to 265 μm . The solid curve is the relationship between spot diameter and response amplitude for a continuous two-dimensional sheet which exhibits a length constant of 18 μm . Inset: photoresponses recorded (from top to bottom, respectively) at 13, 23, 47, 82, 131, 167, 219 and 265 μm spot diameters (curves displaced for illustration). Spots were generated with either an iris diaphragm (for the large spots) or with a plate containing a variety of apertures (for small spots). Spot diameters were calibrated by observing the image cast on the retina with a microscope containing an ocular reticle. Details of the optical system and its calibration are described elsewhere¹⁵.

the predicted signal-to-noise ratio for single photon capture would be increased by a factor of ~ 2.5 . Most importantly, our data show that the spatial properties of *Bufo* red rod responses are well described by an exponential for thin bar-shaped stimuli and a modified Bessel function for circular stimuli. Therefore, our results indicate that a network containing purely passive membrane and coupling resistances can account for the extent of spread of signals between *Bufo* red rods.

This research was supported by the National Eye Institute and the National Institute of Neurological and Communicative Disorders and Stroke, USPHS.

Note added in proof: Detwiler *et al.*²² recently reported that the network of turtle rods possesses some properties of a high-pass filter. In the analysis of our data we have assumed a purely resistive network in modelling the spread of *Bufo* rod signals. It can be shown, however, that under the passive network assumptions used by Detwiler *et al.* (their Fig. 1*b*), the d.c. space constant is still described solely by the resistive network elements if determined from measurements of the peak response amplitude (where $dv/dt = 0$).

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Increase in free Ca^{2+} in muscle after exposure to CO_2

IT IS now established that a change in intracellular free Ca^{2+} forms a vital link in the action of certain hormones and neurotransmitters as well as in excitation–secretion and excitation–contraction coupling^{1–5}. Here we report an increase in intracellular free Ca^{2+} (Ca_i^{2+}), brought about by exposure of muscle fibres to CO_2 . This effect may provide an important clue to the mode of action of CO_2 in muscle cells as well as to a possible regulation of Ca_i^{2+} by changes in intracellular pH (pH_i).

Single muscle fibres from the barnacle *Balanus nubilus* were axially injected with the calcium-sensitive photoprotein aequorin⁶, prepared by chromatography from extracts of *Aequorea forskalea*⁴. Usually a period of 2 h was allowed for uniform distribution of the photoprotein internally, after which the light was recorded with an EMI photomultiplier tube (9634A), run at -900 V, with the output (time constant 10 ms) displayed on a pen recorder. The fibres were bathed in artificial seawater (ASW), composition (mM) NaCl 510, KCl 12.9, CaCl_2 11.8, MgCl_2 23.6, NaHCO_3 2.6 and TES 2.0 at pH 7.1 and temperature 17 – 23°C .

If the ASW was replaced with ASW which had been equilibrated with either 5, 10 or 100% CO_2 so that the values of the saline pH were 6.4, 6.1 and 5.0 respectively, the aequorin light emission increased over a period of 10–20 min, reaching in 100% CO_2 a new steady level which was 5–25 times (nine fibres) the initial resting value (Fig. 1*b–d*). This response to CO_2 was observed immediately after aequorin injection, although the rate at which the steady level was achieved was slower than after the equilibration period of 2 h. If the pH of 100% CO_2 -ASW was adjusted to 6.9 by addition of 100 mM NaHCO_3 (replacing NaCl) there was still a marked increase in aequorin light emission. Application of an ASW at pH 5.0 (buffered with 10 mM NaH_2PO_4 or 10 mM Tris-maleate) produced relatively little change (Fig. 1*a*) in light emission over 20 min (in some fibres a small increase and in others a decrease in light was observed, the maximum change being 0.3 times the resting light). These last two observations suggested that it was either a direct action of CO_2 or a change in pH_i that initiated the aequorin light response.

The fact that CO_2 does readily change pH_i in these and other cells is well documented^{7–10}. Application of 100% CO_2 -ASW produces a change in pH_i of about 1 unit from 7.2 to 6.2 in crustacean muscle fibres after a period of 10–15 min^{7,8}, whereas phosphate-ASW (pH 5.0) produces a change in pH_i of only about 0.2 pH units over the same period⁷. We have also determined that the increase in light emission seen in CO_2 -ASW was not brought about simply by a direct action of either H^+ or CO_2 on the apparent affinity of aequorin for Ca^{2+} . First, in a synthetic internal solution having the following composition (mM) KCl 150, NaCl 20 and MgCl_2 5, with added Ca^{2+} about 60×10^{-6} , at pH 7.1 with K_2CO_3 (Johnson Matthey) there was no increase in the aequorin light emission after equilibration with 100% CO_2 (pH 5.0 finally) and the records in fact indicate a small inhibitory effect which would be expected from the known values of the apparent binding constants of these ions to the photoprotein at

these different pH values¹¹ (Fig. 1e–g). *In vivo*, the pH_i change would be less than occurred in this assay (see above). Second, the increase in light emission was also accompanied by an increase in isometric tension development (Fig. 1h), which was equivalent to about 0.6% of the maximum tension, P_0 , for this preparation (49 N cm⁻²)¹⁴.

All these experimental observations strongly suggest that the increase in aequorin light emission in CO₂-ASW does in fact represent an increase in free Ca_i²⁺. Assuming that the free Ca_i²⁺ is proportional to (aequorin light)^{0.4} (ref. 11), and that the change in Ca_i²⁺ is uniform within the fibre at the peak of the response, the increase in free Ca_i²⁺ by 100% CO₂ is calculated to be approximately two to four times, increasing from 70–100 nM (refs 12 and 13) to 140–400 nM, respectively.

It was important to determine whether this increase in free Ca_i²⁺ resulted from either a stimulation of calcium influx across the surface membrane or a release from internal stores or a combination of both processes. Replacement of ASW with OCa-ASW (Na replaced) reduced the aequorin light response in 100% CO₂ to 7–27% ($n=6$) of the control response in 100% CO₂-ASW, after 12–50 min in OCa-ASW (Fig. 2) and the

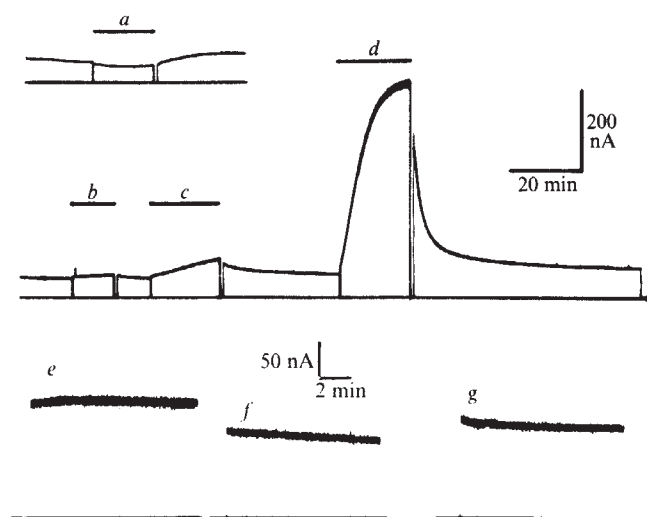


Fig. 1 a–d, Effects of extracellular pH and CO₂ on aequorin light emission from a single muscle fibre in ASW at pH 7.1 TES (N-Tris[hydroxymethyl]-methyl-2-aminoethanesulphonic acid) a, ASW at pH 5.1 (10 mM Na₂HPO₄); b, c and d, ASW equilibrated with 5, 10 and 100% CO₂ at final pH 6.4, 6.1 and 5.0, respectively. Baseline is zero light emission. Ambient light 5 nA. Fibre membrane potential (E_m) –51 mV. e–g, Effects of pH and CO₂ on aequorin light emission from a simulated muscle solution (see text). e and g, controls at pH 7.1; f, after equilibration with 100% CO₂ at final pH 4.8. Each trace shows a separate assay, and starts 3 min after 0.3 μ l saturated aequorin solution was injected into each 50- μ l aliquot and rapidly mixed. Ambient light 2 nA. h, Isometric tension (i) and aequorin light emission (ii) from a single muscle fibre, showing response to 100% CO₂-ASW at pH 5.0. Tension was recorded with an RCA mechanoelectric force transducer connected to a bridge circuit and Devices pen recorder⁴. Fibre diameter 1,150 μ m, E_m –48 mV. Ambient light 5 nA. Calibration for tension 1 mN = 0.2% P_0 .

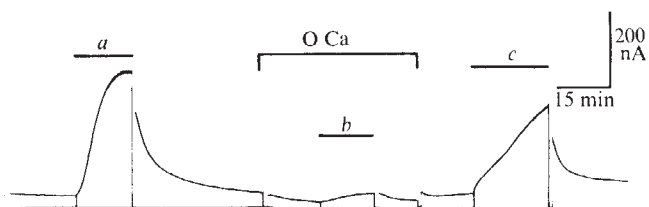


Fig. 2 Effects of OCa (Na substituted) saline on the response to CO₂ of aequorin light emission from a single muscle fibre (E_m –51 mV). a and c, 100% CO₂-ASW; b, 100% CO₂-OCa; final pH both 5.0. Ambient light 5 nA. Note the reduction in resting light emission in OCa-ASW (see ref. 13).

tension response was also abolished. This result seemed to support the idea that a stimulation of Ca_i²⁺ influx was involved in the response to CO₂. However, measurement of the resting ⁴⁵Ca²⁺ influx across the muscle membrane using the intracellular scintillator probe^{14,15} showed that the unidirectional Ca²⁺ influx was reduced in 100% CO₂-ASW by 40% over a 20-min period (three fibres) (Fig. 3a), whereas in phosphate-ASW (pH 5.0) there was no effect over a similar time (three fibres). These fibres had first been injected with 1 M K₂EGTA solution giving a final intracellular concentration of 11–18 mM per kg in order for the probe to assess accurately the intracellular ⁴⁵Ca²⁺ concentration.

The Ca²⁺ efflux, measured after axial injection of ⁴⁵Ca²⁺ (ref. 16), indicated that in 100% CO₂-ASW there was an average reduction of 53% (three fibres) (Fig. 3b), and in phosphate-ASW (pH 5.0) there was an average reduction of 36% (three fibres) in the rate of Ca²⁺ loss from the cell. Thus, if the Ca²⁺ influx and the Ca²⁺ efflux are approximately equal, then the effects of 100% CO₂ would be to produce a net Ca²⁺ influx equivalent to 13% (about 0.1 pmol cm⁻² s⁻¹) of the resting unidirectional influx. The failure of ASW-phosphate (pH 5.0), which would produce a larger net influx equivalent to 36% of the resting unidirectional influx, to increase the free Ca_i²⁺ in a similar way to the effect of CO₂-ASW argues against the smaller net influx of Ca²⁺ observed in CO₂-ASW being by itself responsible for the increase in free Ca_i²⁺. Also, external La³⁺ (2 mM), known to inhibit both the resting Ca²⁺ influx and efflux^{14,17,21} as well as the increase in free Ca_i²⁺ which occurs in Na-free ASW in these fibres¹⁷, failed to affect significantly the CO₂-induced increase in free Ca_i²⁺ (the mean response to CO₂ in the presence of La³⁺ was 93% of the control response in ASW ($n=3$)).

It seemed possible that changes in membrane potential, which have been reported to occur in muscle fibres exposed to CO₂-ASW^{8,18} could have been releasing Ca²⁺ from internal Ca²⁺ stores within the sarcoplasmic reticulum (SR). Measurements with an axial, intracellular electrode (100 μ m tip) filled with KCl (3 M) showed that 100% CO₂-ASW produced a slow depolarisation of 10–15 mV over 20 min (seven fibres). Depolarisations of similar amplitude and duration, produced in the same fibres by 60 K⁺-ASW, resulted in either transient increases in light emission which were less than 10% of the increases observed with 100% CO₂-ASW, or no increases at all.

In conclusion, it seems that external CO₂ application results in an increase in free Ca_i²⁺ in these muscle fibres and that this cannot readily be attributed to either a net Ca²⁺ influx alone or to a depolarisation of the surface membrane, although a CO₂-induced depolarisation may contribute up to 10% of the observed increase in aequorin light emission. The requirement of the response to CO₂ for extracellular Ca²⁺, as shown by the effects of Ca²⁺-free saline, means that the small net Ca²⁺ influx induced by CO₂-ASW should not be ruled out entirely as a possible cause of the response. If, for example, the CO₂-induced reduction in pH_i also impairs the functioning of the Ca²⁺-ATPase pump in the membrane of the SR, then a small net influx of Ca might lead to an increase in free Ca_i²⁺. There is evidence from frog skinned muscle fibres that the Ca²⁺ loading of the SR at physiological free Ca²⁺ levels is decreased by a reduction of pH from 7.0 to 6.2 (ref. 19).

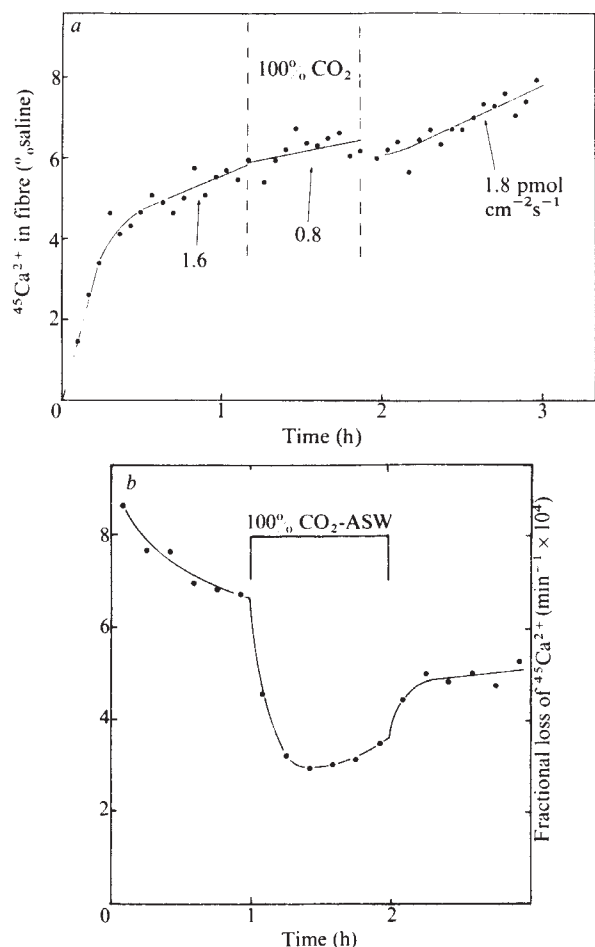


Fig. 3 *a*, Effect of CO_2 on $^{45}\text{Ca}^{2+}$ influx, measured in a single muscle fibre using an intracellular scintillator probe. $^{45}\text{Ca}^{2+}$ concentration in muscle fibre (as percentage extracellular $^{45}\text{Ca}^{2+}$ concentration) is plotted against time of immersion in saline (0.1 mCi ml^{-1}); ASW ($\text{pH } 7.1$), 100% CO_2 -ASW ($\text{pH } 5.0$). Values of the influx ($\text{pmol cm}^{-2} \text{s}^{-1}$), calculated from the slope of the line drawn by linear regression analysis, are shown. Initial fast phase represents filling of extracellular 'cleft' system^{14,15}. Intracellular EGTA 14 mM per kg . $E_m = -47 \text{ mV}$; diameter $1,240 \mu\text{m}$. *b*, Effect of CO_2 on residual $^{45}\text{Ca}^{2+}$ efflux from single muscle fibre which had been axially injected with $^{45}\text{CaCl}_2$ + TES ($\text{pH } 7.1$). $^{45}\text{Ca}^{2+}$ appearing in external ASW was counted by liquid scintillation¹⁶. Fractional loss of $^{45}\text{Ca}^{2+}$ ($\text{min}^{-1} \times 10^4$) plotted against time after injection. The pH of 100% CO_2 -ASW was 5.0 . $E_m = -48 \text{ mV}$.

A second possible mechanism for the CO_2 response is a stimulation by the reduction in pH of a Ca^{2+} -dependent release of Ca^{2+} from the SR, similar to the type which has been proposed to occur in the normal E-C coupling in skeletal muscle fibres²⁰. Relevant to this is the finding that exposure of barnacle muscle fibres to OCa-ASW results in a complete loss of the electrically evoked calcium transient and tension response within 10–15 min (C. C. A. and R. Burles, unpublished). This is thought to reflect a requirement of the E-C coupling process itself for extracellular Ca^{2+} , as the caffeine-releasable internal stores of Ca^{2+} are still reasonably intact at this time²¹ and the amplitude of the extra Ca^{2+} influx which occurs during electrical stimulation in normal saline is insufficient to account for the observed tension development^{14,15}, even if one assumes that all the extra Ca^{2+} binds directly to the contractile proteins.

A third possibility is that there is displacement of Ca^{2+} by H^+ either from some organelle other than the SR or from Ca^{2+} -binding proteins in the sarcoplasm. In this case the effect of OCa-ASW on the CO_2 response could be explained by a ready depletion of this labile intracellular Ca^{2+} store, following the

rapid decline in free Ca_i^{2+} seen in these circumstances (see Fig. 2).

Evidence demonstrating that it may be a reduction in pH , and not the increase in intracellular CO_2 concentration which is the stimulus for the increase in free Ca_i^{2+} was obtained by injecting muscle fibres with various buffer solutions (1 M KH_2PO_4 /0.05 M Na_2HPO_4 , 1 M HEPES or 1 M Tris-maleate). Solutions of $\text{pH } 5.0$ – 5.5 transiently increased the aequorin light emission by up to 10 times, whereas solutions at $\text{pH } 7.2$ did not.

Interestingly, aequorin light emission from squid giant axons is reduced by 5% CO_2 -ASW, and this has been attributed solely to changes in Ca^{2+} fluxes across the axon membrane^{22,23}. The opposite effect of this level of CO_2 on barnacle muscle may mean that in muscle, Ca^{2+} is being released from an intracellular source which is absent in nerve.

This work was supported by a grant from the MRC.

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Received 22 March; accepted 26 July 1978.

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Requirements for therapeutic inhibition of sickle haemoglobin gelation

KINETIC studies on haemoglobin S gelation have shown that following initiation of the reaction there is a pronounced delay before the appearance of the polymer^{1–5}. The delay time is extremely sensitive to physiological variables, such as total haemoglobin concentration and fractional saturation with ligand, and moderately sensitive to pH , salt concentration and temperature (near 37°C)^{1,5–10}. Changes in these variables which decrease the delay time seem to be associated with increased clinical severity of sickle cell disease^{1,11}. These findings have been explained by a pathophysiological mechanism in which a decrease in the delay time of intracellular gelation, relative to the capillary transit time, increases the probability of intracapillary sickling; blockage of the microcirculation is then possible, with consequent tissue anoxia and organ damage. An increase in the delay time, permitting cells to escape the narrow capillaries before gelation has begun, is therefore predicted to result in amelioration of the disease^{1,11}. We present here results of kinetic experiments designed to help answer the question: how much must the delay time be increased to obtain a given therapeutic effect in patients?

The gelation kinetics of haemoglobin S were investigated in mixtures with normal adult haemoglobin (A) or fetal haemoglobin (F). Increased proportions of these normal haemoglobins, if homogeneously distributed in the red cell population, are known to be associated with decreased clinical severity¹²⁻¹⁶. By correlating the delay time data on specific haemoglobin mixtures with the data on the associated clinical course, we obtained a quantitative estimate of the increase in the *in vitro* delay time required to produce a particular clinical status. These results, together with other kinetic data, were then used to evaluate various therapeutic strategies.

The kinetic results reported here were derived from temperature-jump experiments in which the initiation of gelation was instantaneous relative to the delay time⁷. If initiation is not instantaneous, the observed delay will always be longer, this effect increasing with decreasing delay time. In such conditions, the increases in delay time will be smaller than those reported here. Zarkowsky and Hochmuth⁹, for example, found an increase of only 35-fold for the delay time of morphological sickling of A/S (heterozygous) trait cells relative to S/S (homozygous) cells. Kinetic studies on sickle cells by Rampling and Sirs²⁵ suggest that deoxygenation was not sufficiently rapid to resolve the delay time for S/S cells in the Zarkowsky and Hochmuth experiments. We point out, however, that deoxygenation *in vivo* may also not be fast relative to the delay time, so that experiments designed to mimic this situation will show a decreased sensitivity of the delay time to solution variables.

Figure 1 shows the delay time for the thermally induced gelation of mixtures. The effect of deoxyhaemoglobin F on the delay time is considerably larger than the effect of deoxyhaemoglobin A. This result is expected from previous equilibrium and minimum gelling concentration data, which showed that deoxyhaemoglobin F has a larger inhibitory effect than deoxyhaemoglobin A^{6,7,21-24}. A detailed experimental and theoretical analysis of both the kinetic and equilibrium data will be presented elsewhere²⁰.

In the double heterozygous conditions of sickle/ β^+ -thalassaemia, red cells contain about 15–30% haemoglobin A¹². In mixtures of these compositions we measured a 10–100-fold increase in delay time. Although, as in homozygous sickle cell disease, the clinical severity is quite variable, S/ β^+ -thalassaemia is in general less severe than S/S disease^{12,13}. Our kinetic results suggest, then, that some therapeutic effect might result if the delay time of gelation were increased by a factor of 10–100. In the double heterozygous condition of sickle/hereditary persistence of fetal haemoglobin (S/HPFH), red cells contain about 20–30% haemoglobin F^{12,14}. Such mixtures increase the delay time 10³–10⁴-fold. Since this condition produces a much milder clinical course than S/S disease^{12,14}, increasing the delay time by a factor of 10³–10⁴ can reasonably be expected to have a major therapeutic effect in patients. Finally, in sickle cell trait, red cells contain ~60% haemoglobin A and 40% haemoglobin S^{13,15}. The increase in delay time found for a mixture of this composition is ~10⁶-fold. Patients with sickle cell trait rarely show any clinical manifestations¹⁶. Increasing the delay time, then, by about a factor of 10⁶, could be expected to produce a 'cure' of S/S disease.

Three different strategies to increase the delay time of intracellular gelation have been suggested. One is to directly inhibit polymerisation with molecules that competitively bind to sites of intermolecular contact. A second is to inhibit polymerisation indirectly by increasing the haemoglobin oxygen affinity, thereby increasing the fractional saturation in capillaries. A third strategy, suggested by previous kinetic studies, is to decrease the intracellular haemoglobin concentration^{1,11}.

We can estimate the requirements for each strategy. The decrease in intracellular haemoglobin concentration, Δc , required to produce a given increase in the delay time, t_d , can be directly calculated from the measured concentration dependence: $d \log t_d / d \log c = -(35-55)$ (refs 1, 6, 20). In the limiting case where a molecule binds to a single site and completely prevents incorporation into the polymer, the frac-

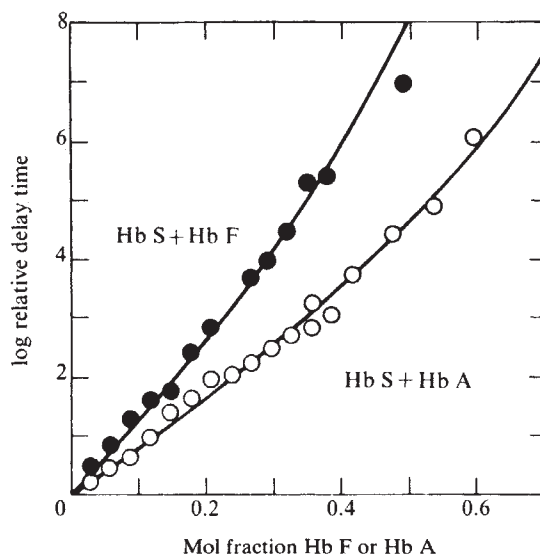


Fig. 1. Effect of deoxyhaemoglobins A and F on the delay time of thermally induced gelation. The relative delay time is defined as the ratio of the delay time for the mixture, t_d , to the delay time for deoxyhaemoglobin S alone, $t_d(S/S)$, at the same total haemoglobin concentration. To obtain relative delay times over seven decades at the same total haemoglobin concentration and temperature (286 g l⁻¹ and 23.4 °C for S-F mixtures, 270 g l⁻¹ and 23.0 °C for S-A mixtures), delay times were measured as a function of total haemoglobin concentration and temperature using a turbidity technique⁷ and then extrapolated to the same values. All mixtures were initially prepared in the oxy form to permit complete hybridisation¹⁷⁻¹⁹. The curve through the S-F mixture points is theoretical, and is obtained from the equation $\log(t_d/t_d(S/S)) = -s \log(1 - X_F)^2$, which assumes that the molecular species— $\alpha_2\beta_2^S$, $\alpha_2\gamma_2$ and $\alpha_2\gamma\beta^S$ hybrid—are present in a binomial distribution¹⁷⁻¹⁹, and that only the $\alpha_2\beta_2^S$ species polymerises. A least-squares fit to the data gives $s = 13.5$. Details of the experimental procedures and data analysis will be presented elsewhere²⁰.

tional saturation of the site with inhibitor, x , can be calculated from the relation: $d \log t_d / d \log(1 - x) \approx -10$. This derivative is an average of values obtained from experiments in solutions partially saturated with carbon monoxide and from the S-F mixture data in Fig. 1^{20,26}. It is smaller than the corresponding derivative for the concentration dependence because the inhibited molecule, while incapable of being incorporated into the polymer, still occupies space in the solution and thereby increases the kinetically effective concentration of the uninhibited, polymerising molecules^{20,26-29}. Oxygen affinity calculations are more speculative, as the only relevant kinetic result is $d \log t_d / dy \approx 10$ ($0 < y < 0.6$), where y is the fractional saturation of haemoglobin S with carbon monoxide^{7,8}. Assuming that the perturbation responsible for the change in oxygen affinity alters neither the degree of cooperativity nor the delay time for the completely deoxygenated molecule, we can use the result for carbon monoxide to calculate the minimum decrease in p_{50} required to produce a given increase in delay time. Table 1 summarises the results of the above calculations. As solubility is a frequently used assay for gelation, and is related to the delay time by $d \log t_d / d \log c_s = 30-40$ (refs 7, 26, 30), the increase in solubility corresponding to the delay time increase is also shown.

Table 1 suggests that the values of x , Δc and Δp_{50} required to produce a 'cure' of S/S disease are large and will be difficult to achieve. To obtain 75% saturation of a perfect inhibitory site, and simultaneously maintain a low free inhibitor concentration to minimise toxicity, a molecule with a high binding constant is required. A decrease in intracellular haemoglobin concentration of 75–110 g l⁻¹ might be accomplished with a reduction

Table 1 Clinical course, gelation delay time and requirements for methods of inhibition

Condition	Clinical course relative to S/S disease	Red cell composition			t_d	$\frac{c_s}{c_s^0}$	x	Δc (g l ⁻¹)	Δp_{50} (mm Hg)
		X_S	X_A	X_F	$t_d(S/S)$	$\frac{c_s}{c_s^0}$			
S/ β^+ -thal.	Less severe	0.85–0.7	0.15–0.3	0	10–10 ²	1.06–1.17	0.20–0.37	15–40	>4–7
S/HPFH	Much less severe	0.8–0.7	0	0.2–0.3	10 ³ –10 ⁴	1.19–1.26	0.50–0.60	40–80	>10–13
A/S trait	No disease	0.4	0.6	0	10 ⁶	1.4–1.6	0.75	75–110	>17

X_i values ($i = \text{Hb S, Hb A, Hb F}$) are mol fractions; $t_d/t_d(S/S)$ is the ratio of the mixture delay time for each composition to the delay time for pure deoxyhaemoglobin S at the same total haemoglobin concentration and is obtained from the data in Fig. 1; c_s/c_s^0 is the ratio of deoxyhaemoglobin S solubility in the presence of inhibitor to deoxyhaemoglobin S solubility without inhibitor; x is the fractional saturation of the inhibitory site required to produce the delay time increase; Δc is the required decrease in intracellular haemoglobin concentration, assuming a mean intracellular concentration of 340 g l⁻¹ in S/S disease; Δp_{50} is the required decrease in partial oxygen pressure at 50% saturation with oxygen. For a given increase in the delay time the minimum Δp_{50} can be calculated from the experimental result on solutions partially saturated with carbon monoxide that $d \log t_d/dy = 10^{7.8}$, and from the Hill equation, $\log(y/(1-y)) = n \log(p/p_{50})$. It is assumed that $n = 2.6$ and $p_{50} = 26$ mm Hg for haemoglobin S during the delay period, where a negligible amount of polymerisation has occurred.

in haemoglobin synthesis, by, for example, introducing an iron deficiency (the ideal result would be a 'switch' from sickle cell disease to a much less severe anaemia)^{11,31}. An equivalent increase in cell volume (22–32%) would be preferable, as it would presumably result in less interference with oxygen delivery. Increasing oxygen affinity is the most difficult strategy to evaluate. A realistic calculation would require detailed considerations of the kinetics of intracapillary deoxygenation. There is, moreover, insufficient data to evaluate the influence of homeostatic responses that maintain oxygen delivery. A decrease in tissue p_{O_2} to increase oxygen extraction, would, for example, partially buffer the inhibitory effect of an increase in oxygen affinity³². Note, however, that complete suppression of 2,3-diphosphoglycerate synthesis could lower the p_{50} by as much as 10 mm Hg^{33,34}. Finally, we emphasise that the calculations in Table 1 consider only changes in single variables. Both molecular and physiological responses to a particular perturbation could result in simultaneous changes in all three. However, the comparisons in Table 1 suggest that therapeutically significant kinetic inhibition of intracellular gelation could be accomplished by any one of the three approaches.

Many gelation inhibitors have been proposed as specific drugs for sickle cell disease³⁵. The results in Table 1 should provide a useful framework for assessing the therapeutic potential of these inhibitors, particularly in analysing the problem of gelation inhibition versus toxicity. Because very large doses of any single drug would be necessary to obtain a therapeutic effect in patients, it will probably be necessary to employ several drugs simultaneously, taking advantage of the multiple methods by which the delay time of intracellular gelation can be increased.

H.R.S. is the recipient of a NRS award from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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Received 8 May; accepted 10 July 1978.

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Initiation activity of EMC virus RNA, binding to initiation factor eIF-4B and shut-off of host cell protein synthesis

PICORNAVIRUSES infecting susceptible animal cells inhibit synthesis of host proteins and direct the cells to synthesise viral proteins¹. The mechanism for the shut-off of host cell protein synthesis is not understood, although several experiments indicate that initiation of host protein synthesis is specifically inhibited¹. When initiation of protein synthesis is reduced by exposing cells infected by picornaviruses to hypertonic medium, synthesis of viral proteins is greatly favoured over that of host proteins^{2,3}. This suggests that viral templates are more successful in initiation than host templates³. Competition between picornavirus RNA and host mRNAs has been shown directly by *in vitro* translation experiments^{4,5}. When these RNAs are added together in saturating amounts to a cell-free protein-synthesising system, viral RNA is preferentially translated over host mRNA^{4,5}. This competition can be relieved by addition of initiation factor eIF-4B (previously designated IF-M₃)⁶. This has suggested that eIF-4B may be present in limiting amounts in the translation assays, and that viral and host templates compete for available factor⁶. This observation is somewhat surprising, as the 5'-terminal cap sequence m⁷G⁵pppXmp... is involved in the binding of mRNA to eIF-4B, and picornavirus RNA is not capped (see refs 7 and 8). It has been found that other nucleotide sequences outside the cap participate in this reaction⁹, but the presence of 5'-terminal m⁷G in mRNA promotes interaction

with eIF-4B and the initiation process^{7,8}. Therefore, templates with a 'capped' 5'-terminal nucleotide bind to ribosomes more rapidly than their unmethylated counterparts^{10,11}. We have shown previously that eIF-4B interacts with the RNA of encephalomyocarditis (EMC) virus, a picornavirus, in a nitrocellulose filter binding assay⁷. It has also been shown that eIF-4B is required for *in vitro* translation of EMC virus RNA¹². In the present report we have examined the basis for the competition between EMC virus RNA and a cellular mRNA at the molecular level. EMC virus RNA binds to ribosomes at a rate similar to that of capped mRNAs, suggesting that features other than the 5'-terminal m⁷G are responsible for the binding of this viral RNA. By measuring the relative affinity of globin mRNA and EMC virus RNA for eIF-4B, we have found that the viral RNA has a greater affinity for this initiation factor than globin mRNA. This high affinity for eIF-4B could explain the fast rate of initiation of the viral RNA and the shut-off of host cell protein synthesis after picornavirus infection.

The rate of binding of EMC virus RNA to ribosomes was examined in cell-free systems prepared from HeLa cells and reticulocyte lysate¹³. Labelled EMC virus RNA obtained from virions or from infected cells formed initiation complexes by binding to ribosomal subunits (Fig. 1). As was previously described^{7,10}, the cell extracts were incubated for 2 min with the inhibitor of elongation sparsomycin before addition of viral RNA. In the presence of sparsomycin, ribosomes were stuck to the initiation site of cellular mRNA. Therefore, the cellular mRNA was effectively blocked from reinitiating. This procedure avoided competition between the trace amount of the highly radioactive viral RNA added and the larger amounts of cellular mRNA present in the extracts, and enabled us to measure the kinetics of binding of viral RNA to ribosomes without interference from competitor mRNA species (Fig. 2).

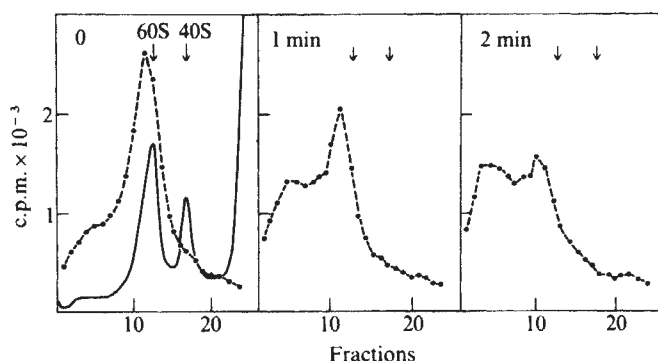


Fig. 1 Binding of EMC RNA to HeLa cell ribosomes (●, c.p.m. $\times 10^{-3}$). Labelled EMC virus was obtained from infected HeLa cells incubated with ³H-uridine, and the viral RNA purified as described previously⁷. Cytoplasmic extracts were prepared from HeLa cells as described previously¹³. Each incubation contained in a final volume of 0.1 ml: 70 μ l cell extract, 5 μ l 2 mM sparsomycin, 1 μ g reticulocyte ribosomal RNA, 20 μ l of the energy mix for protein synthesis described previously¹², amino acids at 0.05 mM, K OAc at 0.12 M and Mg(OAc)₂ at 2.5 mM final concentrations. After 2 min incubation at 30 °C, 5 μ l EMC ³H-RNA (20,000 c.p.m.) were added for the time indicated. The incubations were terminated by adding 4 volumes ice-cold 0.5 M NaCl, 30 mM Mg OAc₂ and 20 mM HEPES-KOH, pH 7.4, and applied to 15–30% sucrose gradients in the same buffer. After 17 h centrifugation at 23,000 r.p.m., the gradients were fractionated through a recording spectrophotometer. An identical A₂₆₀ profile (solid line) was obtained for all gradients and is reported in the first panel only. The gradient fractions were counted after addition of 9 ml Scintiverse (Fisher). In the left panel, the incubation has been terminated immediately after addition of the RNA and the EMC RNA is not bound to ribosomes. In the other panels, the binding of EMC RNA to ribosomes is shown by the appearance of a peak sedimenting faster than the unbound EMC RNA. To determine the amount of bound EMC RNA as a per cent of input RNA (Fig. 2), the areas of the peaks corresponding to free and bound EMC RNA were measured with a Du Pont 310 Curve Resolver.

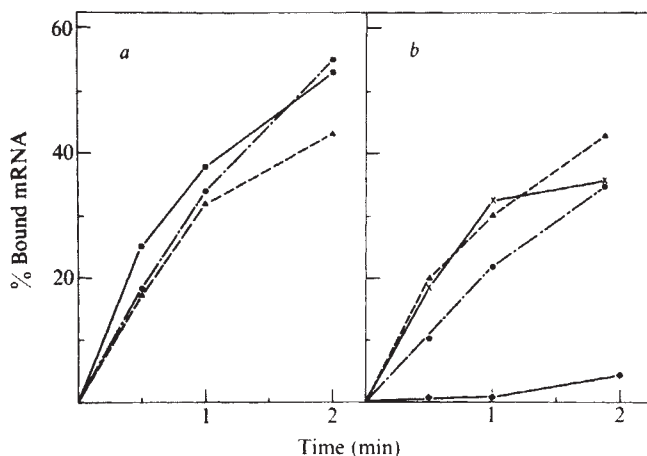


Fig. 2 Rate of binding to ribosomes of EMC RNA and of other viral mRNAs, in HeLa cell extract (a) and in a reticulocyte lysate (b). EMC ³H RNA was obtained from virions (●) as described previously⁷, or from infected HeLa cells (■) according to Fernandez-Munoz and Lavi²⁸, as follows: the cells were infected at room temperature as outlined previously⁷; after 30 min, actinomycin D was added to a final concentration of 5 μ g ml⁻¹. After 90 min, 5 μ Ci ml⁻¹ ³H uridine were added and the incubation continued for 2 h. The cells were collected, washed and homogenised as described previously¹³. The cell extract was made 0.5% in NP-40 and 30 μ g ml⁻¹ in dextran sulphate, centrifuged for 5 min to remove the nuclei, and adjusted to a final concentration of 20 mM EDTA and 1% sodium dodecylsulphate. The 35S EMC RNA was purified by three successive centrifugations on 15–30% sucrose gradients as described previously⁷. The EMC RNA had a specific activity of about 200,000 c.p.m. per μ g RNA. Other viral mRNAs were prepared as described previously for vesicular stomatitis virus polysomal mRNA⁷ (▲), and for methylated (×) and unmethylated (○) vaccinia mRNA¹¹. The rate of binding of the different RNAs was measured as described in Fig. 1. The input of VSV mRNA was 10,000 c.p.m. per incubation. The binding assays with vaccinia mRNA have previously been described.¹¹

To resolve EMC virus RNA bound to ribosomes from unbound viral RNA, reaction mixtures were centrifuged through sucrose gradients containing 0.5 M NaCl (Fig. 1). In these conditions, single ribosomes were dissociated, whereas initiation complexes were stable¹⁰. Moreover, nonspecific association of cytoplasmic proteins with EMC virus RNA was reduced, allowing a better separation of the RNA bound to ribosomes from unbound RNA¹⁴.

In a reaction kept at 0 °C most of the EMC virus RNA not bound to ribosomes sedimented at about 70S (Fig. 1). After 1 min incubation, a peak sedimenting at about 10S was seen, and after 2 min incubation this peak was approximately equal in height to the peak of unbound EMC virus mRNA. As these peaks were incompletely resolved, it was necessary to use a Du Pont curve resolver to calculate the percentage of input RNA bound to ribosomes (see Fig. 1). This difficulty in determining the percentage of RNA bound to ribosomes was only encountered with the large picornavirus RNA. Binding of one ribosome to this RNA did not increase its sedimentation sufficiently to allow complete separation between bound and unbound RNA, as is the case with smaller mRNAs^{7,11}.

The rate of binding of EMC virus RNA to ribosomes was similar to that of capped viral mRNAs obtained from cells infected with vesicular stomatitis virus (VSV) or transcribed *in vitro* from vaccinia virions^{7,11}. The rate of binding was linear for 1 min and decreased thereafter; very little additional binding was observed after 2 min of incubation (not shown). Unmethylated vaccinia mRNA bound to reticulocyte ribosomes very slowly (Fig. 2b), as previously reported¹¹. This result was also obtained in the HeLa cell-free system (not shown). The rate of binding of cellular histone mRNA was determined in separate experiments (L. A. Weber and C. B., unpublished observations) and was found to be similar to that of the viral mRNAs. Histone mRNA is also capped¹⁵.

These results indicate that the uncapped EMC virus RNA interacts with initiation factors and ribosomal subunits to form initiation complexes at a rate comparable to that of capped cellular and viral mRNAs. As the interaction of mRNAs with initiation factor eIF-4B may play an important part in determining the rate of initiation, we measured the binding of viral RNA and globin mRNA to purified eIF-4B. The relative affinity of an RNA for this factor was determined by competition-binding experiments (Table 1)⁷. Competition-binding is the most sensitive and accurate method for comparing the relative affinity of two mRNAs for a given initiation factor, and in the conditions used only high affinity interactions were measured. This was obtained by reacting eIF-4B with labelled mRNA at low concentrations, such that only 25–30% of the input mRNA was bound (Table 1). Different unlabelled mRNAs were then added to determine their relative effect as competitors in the binding with eIF-4B. The validity of this assay was shown by experiments with globin mRNA (Table 1). The same preparation of globin mRNA purified by oligo(dT)-cellulose chromatography and gradient centrifugation⁷ was either labelled with ¹²⁵I or used unlabelled as a competitor. A 60% binding competition was observed after addition of a 2.9-fold excess of unlabelled mRNA. The increased concentration of mRNA in this reaction favoured its association with eIF-4B compared with the reaction containing unlabelled mRNA only, and consequently the apparent competition was less than the theoretical level expected. However, the important point shown by the experiments of Table 1 is that on a molar basis EMC virus RNA competed with labelled VSV and globin mRNA about 10-fold and 100-fold more effectively than unlabelled globin mRNA. Even on a microgram basis, EMC virus RNA was more effective than globin mRNA in competing with labelled globin mRNA, as 0.06 µg EMC-RNA competed almost as effectively as 0.42 µg unlabelled globin mRNA.

These findings provide direct evidence that eIF-4B was not bound randomly to RNA, in proportion to the total mass of nucleotides, but that the binding was occurring at specific site(s). Additional evidence for this specific interaction was obtained by using ribosomal RNA instead of mRNA as a competitor in the binding reactions and by directly determining the affinity of EMC virus RNA and globin mRNA for eIF-4B. Ribosomal

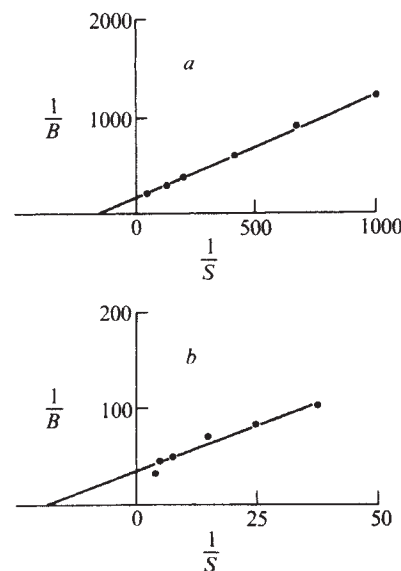


Fig. 3 Binding affinity of EMC RNA (a) and globin mRNA (b) for initiation factor eIF-4B. The binding assays were carried out as described in Table 1, with 0.05 µg eIF-4B per assay and either EMC ¹²⁵I RNA (specific activity 4×10^6 c.p.m. µg⁻¹) or globin ¹²⁵I mRNA (specific activity 1.8×10^6 c.p.m. µg⁻¹). The reciprocals of the bound RNA (1/B) compared with the input RNA (1/S) are shown. Concentrations are in pmol. The dissociation constant (K_D) is given by the intercept of the lines with the abscissa²⁹. The K_D for EMC RNA is 1.43×10^{-10} mol l⁻¹ and that for globin mRNA is 1.08×10^{-9} mol l⁻¹.

Table 1 EMC virus RNA competition for binding of VSV mRNA and globin mRNA to purified reticulocyte initiation factor eIF-4B (IF-M₃)

Competitor RNA	µg	pmol	mRNA bound (c.p.m.)	%
			³ H-VSV mRNA	
None	—	—	3713	—
Globin mRNA	0.25	1.1	2593	30
EMC RNA	0.94	0.3	1612	57
¹²⁵ I globin mRNA				
None	—	—	4702	—
Globin mRNA	0.42	1.8	1874	60
EMC RNA	0.06	0.02	2241	52

Incubations were carried out at 23 °C for 5 min in a 50 µl reaction mixture and contained 20 mM Tris-HCl, pH 7.4, 0.1 M KCl, 1 mM dithiothreitol, 1.6 µg eIF-4B plus 14,620 c.p.m. ³H-VSV mRNA (3.6 pmol) or 0.2 µg eIF-4B plus 14,700 c.p.m. of ¹²⁵I-globin mRNA (0.62 pmol), and amounts of competing unlabelled globin mRNA or EMC RNA as indicated. Reactions were stopped by dilution to 3 ml with cold buffer (20 mM Tris-HCl, pH 7.4, and 100 mM KCl), and mRNA-protein complex formation measured by collection of the complex on a nitrocellulose filter as previously reported⁷. Blanks of 150 c.p.m. for ³H-VSV or 736 c.p.m. for ¹²⁵I-globin mRNA, representing retention of radioactivity on the filter in the absence of added eIF-4B, were subtracted from each value. Globin mRNA was iodinated with ¹²⁵I-Iodide according to a modification²⁶ of the procedure of Commerford²⁷. A molecular weight of 2.3×10^5 has been estimated for rabbit globin mRNA³⁰.

RNA was found to be a poor competitor of the binding of mRNA to eIF-4B as only 100-fold greater amounts of rRNA competed appreciably in the reaction (data not shown). The affinity of EMC virus mRNA and globin mRNA for eIF-4B was determined by reacting a constant amount of initiation factor with decreasing amounts of RNA. The results are shown as a double-reciprocal plot in Fig. 3. The experimental points, and in particular those presumably measuring high affinity interactions at high RNA dilutions, fall on a line. This enabled us to measure the dissociation constants of complex formation by a graphic method (see legend to Fig. 3) and to establish that the affinity of EMC virus RNA for eIF-4B is approximately one order of magnitude higher than that of globin mRNA.

The present study is the first report of a determination of binding constants (K_D) for mRNA/eIF-4B interaction. Note that these K_D values are averages and that no assumption has been made in their calculation regarding specific sequences involved, number of binding sites, or configurational properties of the RNA or protein.

However, the significance of the binding of mRNA to eIF-4B cannot be functionally evaluated as with the presently available assays it is not possible to demonstrate that these complexes are biologically active.

The high affinity of EMC virus RNA for eIF-4B is a property of the viral RNA, which most likely determines its rate of initiation *in vitro* and its ability to compete more successfully than capped mRNAs *in vivo*. An increased rate of initiation has been shown after enzymatic addition of an m⁷G⁵p cap to the uncapped RNA of satellite tobacco necrosis virus RNA¹⁶. Addition of a cap also increases the affinity of this viral RNA for eIF-4B⁹. Picornavirus RNA competes more successfully than not only host mRNA but also other capped viral RNAs, such as VSV mRNA. In cells infected with VSV and superinfected with poliovirus, host and VSV mRNAs are translated poorly compared with poliovirus RNA^{17,18}. In these conditions, synthesis of VSV mRNA continues, but this RNA does not associate with polysomes¹⁸. Although other interpretations are possible, these observations could be explained by the high affinity of picornavirus RNA for eIF-4B reported here. The

structural basis for this high affinity is not known. Picornavirus RNA has a rather unique structure with a protein covalently bound to the 5'-terminal nucleotide^{19,20}. However, it seems unlikely that this protein plays a part in the interaction with eIF-4B and in the competition between picornavirus and host mRNA, as the viral mRNA synthesised in infected cells does not have a protein bound to the 5'-terminal nucleotide^{21,23}. Nucleotide sequences shared by virion RNA and by viral mRNA are presumably involved in the interaction with eIF-4B.

Initiation factors are found bound to 40S ribosomal subunits²⁴, are released after formation of initiation complexes and recycle in order to function catalytically in initiation¹². The high affinity of picornavirus RNA for eIF-4B may interfere with the recycling of this factor. The viral RNA may form a more stable complex with eIF-4B and this may prevent the factor from functioning with host mRNAs. Golini *et al.*⁶ have proposed a similar explanation for their observation that addition of eIF-4B relieves the competition between EMC virus RNA and host mRNA for *in vitro* translation. The present study is consistent with this interpretation in that EMC virus RNA has a significantly higher binding affinity for eIF-4B than globin mRNA, a natural substrate for this initiation factor. However, binding of other initiation factors to EMC virus RNA has been reported⁷, and interaction with these other factors also plays an important part in the translation of this viral RNA²⁵. Therefore, it remains to be established whether the high affinity of picornavirus RNA for eIF-4B by itself accounts for the shut-off of host protein synthesis in virus-infected cells.

We thank Dr W. C. Merrick for purified initiation factor eIF-4B. This work was supported by NIH grants to C.B. and D.A.S., the Irma T. Hirsh Charitable Trust of New York and also NIH Research Career Development Award to D.A.S.

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Received 21 February; accepted 17 July 1978.

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Does ultraviolet light enhance postreplication repair in mammalian cells?

D'AMBROSIO AND SETLOW reported an apparent enhancement of postreplication repair in Chinese hamster V79 cells¹. A first exposure of cells to ultraviolet (UV) light was said to make it possible, after re-exposure to UV, for the cells to join the DNA together to form high molecular weight DNA more rapidly than if the cells had not been previously exposed. They irradiated cells with 2.5 J m⁻² of UV 2 h before a second exposure to 5.0 J m⁻², and then pulse-labelled the cells with ³H-thymidine before chasing for 1 h. When analysed by alkaline sucrose gradient velocity sedimentation, the DNA of these cells showed a profile of radioactivity very different from that of cells irradiated only once with 5.0 J m⁻² before pulse-labelling and chasing. For cells irradiated twice the peak of labelled DNA was at a position corresponding to a higher molecular weight than the peak for cells irradiated once. I report here results confirming the data of D'Ambrosio and Setlow, but use of an alternative method of data presentation suggests that interpretations other than the one assumed by D'Ambrosio and Setlow are equally possible or maybe more likely.

When Chinese hamster cells were irradiated with 2.5 J m⁻² and, 2 h later, with 5.0 J m⁻² of UV light and then pulse-chased, their ³H-labelled DNA showed two peaks of radioactivity on alkaline sucrose gradient profiles plotted as percentage of total counts in each gradient (Fig. 1). One peak (fraction 18) was near the peak exhibited by the DNA of unirradiated cells receiving the same protocol, and the other was at a position of lower molecular weight (fraction 13). Cells treated with 7.5 J m⁻² and then pulse-chased showed a major ³H-labelled DNA peak corresponding to a low molecular weight (fraction 8) and a broad shoulder extending into the position of the peak for cells irradiated with 2.5 + 5.0 J m⁻². The ³H-labelled DNA from cells treated with 5.0 J m⁻² peaked at the same fraction as the minor peak of cells irradiated with 2.5 + 5.0 J m⁻² (fraction 13); a prominent shoulder at high molecular weight (fractions 15–18) was also present. The ³H-labelled DNA from cells treated only with 2.5 J m⁻² exhibited an alkaline gradient profile which was intermediate between that of DNA from controls and DNA from cells treated with 2.5 + 5.0 J m⁻² (Fig. 1). These results confirm the data of D'Ambrosio and Setlow.

D'Ambrosio and Setlow based their interpretation of the data on profiles plotted as percentage of total radioactivity in each gradient. When the same data are presented in a different form another interpretation is possible. Profiles plotted as total radioactivity per fixed number of cells (Fig. 2) demonstrate the inhibition of DNA synthesis caused by each treatment; this information is lacking in graphs plotted as percentage of total radioactivity in each gradient (Fig. 1). Figure 2 shows that the total radioactivity in fractions 18–21 from ³H-labelled DNA of cells irradiated with 2.5 + 5.0 J m⁻² was only slightly higher than the radioactivity in the same fractions from the DNA of cells treated only with 7.5 J m⁻², and lower than that in cells treated only with 5.0 J m⁻². The profile from cells that received only 2.5 J m⁻² shows the greatest amount of radioactivity in these fractions (except for control) but is still severely depressed from the control. When, as in Fig. 2, the data are plotted to show the large inhibitions of DNA synthesis that occur after UV irradiation, the significance of peaks that appear distinctive in Fig. 1 becomes questionable.

The data presentation in Fig. 2 also poses the question, whether the status of DNA replication in cells 2 h after 2.5 J m⁻² of UV is the same as in unirradiated cells? To answer this question, a similar experimental protocol was used but without a chase after the ³H-thymidine pulse. When the ³H-labelled DNA from these pulse-labelled cells was analysed on alkaline sucrose gradients (Fig. 3) their principal peaks were, as expected, at lower molecular weights than unirradiated cells. The ³H-

labelled DNA from cells exposed to $2.5 + 5.0 \text{ J m}^{-2}$ had a minor but significant peak corresponding to a molecular weight even higher than that of the main peak of control ^3H -labelled DNA. A very prominent minor peak at about the same point was also evident in the profile of ^3H -labelled DNA from cells exposed only to 2.5 J m^{-2} at 2.5 h before the pulse with ^3H -thymidine. The appearance of these peaks at high molecular weight is reproducible.

Analysis of profiles of both pulse-labelled and pulse-chased DNA leads to the following conclusions:

(1) Cells exposed to 2.5 J m^{-2} have not recovered from the damage in their DNA by 2.5 h after irradiation; the overall rate

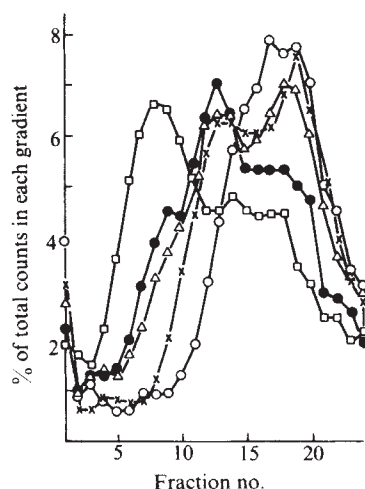


Fig. 1 Alkaline sucrose gradient profiles of ^3H -labelled nascent DNA from Chinese hamster cells. Chinese hamster V79 cells, maintained in this laboratory for several years, were grown in Falcon plastic Petri dishes in Eagle's minimum essential medium (MEM) containing 15% fetal calf serum (Gibco). Exponentially growing cells incubated overnight in $0.01 \mu\text{Ci ml}^{-1}$ of ^{14}C -thymidine (57 Ci mol^{-1} ; Schwarz/Mann) were washed twice with Puck's phosphate-buffered saline G^2 and exposed to various fluences of UV light from a mercury germicidal lamp with an incident intensity of $1.3 \text{ J m}^{-2} \text{ s}^{-1}$. After irradiation, MEM was placed back on the cultures for 30 min, after which it was replaced with MEM containing $20 \mu\text{Ci ml}^{-1}$ of ^3H -thymidine ($4 \times 10^{-7} \text{ M}$). After 10 min of incubation, the radioactive medium was removed. Fresh MEM containing 10^{-4} M thymidine and 10^{-5} M deoxycytidine was added to the cells, which were incubated (chased) for 1 h before the reaction was stopped with ice-cold 0.15 M sodium chloride, 0.015 M sodium citrate (SSC). The cells were washed once with SSC, scraped into it, and a sample containing about 10^5 cells (in less than 0.5 ml) was irradiated with $1,000 \text{ rad}$ of X rays to enhance lysis³ and then added directly to 0.5 ml of lysis solution (0.5 M NaOH, 0.02 M EDTA, 0.1% NP40 [non-ionic detergent, Shell]) on top of 30 ml of preformed $5\text{--}20\%$ alkaline sucrose gradients (0.1 M NaOH, 0.9 M NaCl, 0.01 M EDTA). Lysis proceeded in the dark at 25°C for 30 min and the gradients were then centrifuged in an SW27 rotor (Beckman) at $27,000 \text{ r.p.m.}$ for 3 h or at $12,000 \text{ r.p.m.}$ for 15 h. This gradient system has been calibrated with phage λ DNA and phage T_4 DNA as described⁴. About 25 equal-weight fractions were collected, $100 \mu\text{g}$ of calf thymus DNA were added to each fraction, and all of the DNA was precipitated with ice-cold 6 M HCl plus 6% sodium pyrophosphate. Each fraction was then filtered through GF/C filters (Whatman) presoaked with 4% HClO_4 . The filters were washed sequentially with ice-cold 4% HClO_4 , 70% alcohol, and 100% alcohol and dried; the ^{14}C and ^3H on each filter were determined with a Packard liquid scintillation spectrometer. $\circ$, Unirradiated cells; $\times$, cells irradiated only with 2.5 J m^{-2} at 2.5 h before pulse-labelling; $\triangle$, cells irradiated with 2.5 J m^{-2} at 2.5 h before pulse-labelling and with 5.0 J m^{-2} 0.5 h before pulse-labelling; $\bullet$, cells irradiated only with 5.0 J m^{-2} at 0.5 h before pulse-labelling; $\square$, cells irradiated only with 7.5 J m^{-2} at 0.5 h before pulse-labelling. Sedimentation is from left to right. Note that the ordinate is percentage of total counts in each gradient. The majority of the ^{14}C -labelled parental DNA sedimented to the bottom of the gradient in all cases.

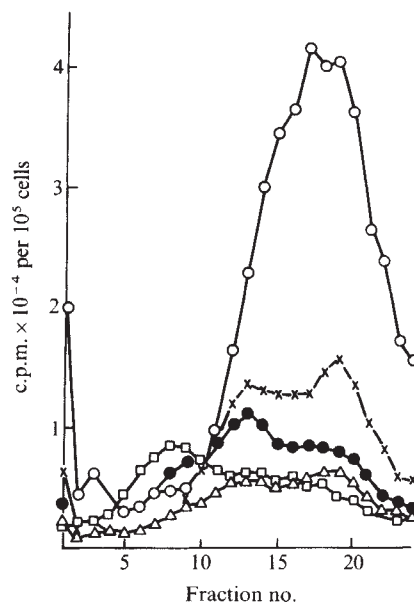
of DNA synthesis is still depressed by 25–50%. More importantly, the status of nascent DNA molecules in these cells is not the same as in controls; although the molecular weight of most nascent DNA molecules in treated cells is lower than in control cells, a significant number of them have a higher molecular weight (fractions 12–14, Fig. 3).

(2) When cells are exposed to 2.5 J m^{-2} of UV and then 2 h later to 5.0 J m^{-2} , 30 min after the second exposure (at the time of pulse-labelling), their nascent DNA molecules more closely resemble the nascent DNA in cells treated only with 5.0 J m^{-2} than in cells treated only with 7.5 J m^{-2} (Fig. 3). The gradient profile of DNA from cells treated with $2.5 + 5.0 \text{ J m}^{-2}$ also resembles that of DNA from cells treated only with 2.5 J m^{-2} , in that a significant amount of nascent DNA has a much higher than average molecular weight after both treatments (peak at fraction 14, Fig. 3). These results imply that 2 h after cells have been exposed to 2.5 J m^{-2} UV, many elongating DNA chains are still temporarily blocked but others have circumvented the lesions and extended to form molecules that are larger than average but still not of parental size. When the cells are subsequently exposed to 5.0 J m^{-2} , these intermediate-sized nascent DNA molecules remain as a source for further chain elongation.

(3) When pulse-chase experiments are performed, the peaks at high molecular weight (fraction 18, Figs 1 and 2) in gradient profiles of DNA from cells treated with 2.5 and 5.0 J m^{-2} probably represent (i) DNA molecules formed by the elongation of the intermediate molecular weight molecules (peak at fraction 14, Fig. 3) observed in pulse-labelled cells and (ii) DNA molecules formed by elongation of low molecular weight molecules (fraction 5, Fig. 3), as in cells exposed to 5.0 J m^{-2} alone. Elongation of intermediate molecular weight molecules accounts for the greater amounts of radioactivity at high molecular weight (fractions 18–21, Figs 1 and 2) in the DNA of cells treated with $2.5 + 5.0 \text{ J m}^{-2}$ over the radioactivity in the same fractions in cells exposed only to 7.5 J m^{-2} .

Thus, the effects of 5.0 J m^{-2} of UV light on DNA synthesis and on maturation of nascent Chinese hamster V79 DNA are different in cells that were irradiated 2 h earlier with 2.5 J m^{-2} than in previously unirradiated cells, but these differences can be explained entirely by the damaging effects of the first exposure, which cause an abnormal distribution of sizes of nascent DNA molecules at the time of irradiation with 5.0 J m^{-2} . These considerations make it unnecessary to invoke the existence of an

Fig. 2 Same data as Fig. 1, except that the ordinate measures total counts per 10^5 cells.



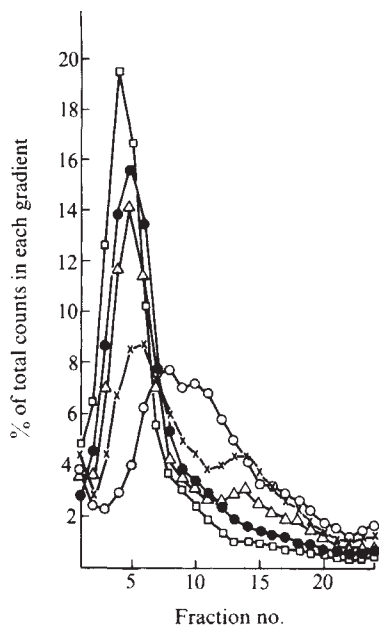


Fig. 3 Alkaline sucrose gradient profiles of ^3H -labelled nascent DNA from Chinese hamster cells. Protocol and symbols the same as Fig. 1, except that the cells were pulse-labelled but not chased before analysis. The reaction was stopped by addition of ice-cold SSC.

enhanced postreplication repair system to explain the altered pulse-chase profiles observed for the DNA of mammalian cells exposed to two irradiations of UV.

I thank Barbara Young for carrying out these experiments and Drs M. Mattern and J. E. Cleaver for help in preparing the manuscript. This work was supported by the US Department of Energy.

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Received 15 May; accepted 24 July 1978.

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Identification of the haem ligands of cytochrome b_{562} by X-ray and NMR methods

X-RAY crystallography and nuclear magnetic resonance (NMR) are the two most powerful physical techniques for studying macromolecular structure. They are often used to complement one another in the study of a particular molecule. Crystallographic results have been of tremendous help in the NMR peak assignments which are subsequently used to obtain structural information in solution^{1,2}. We report the combined use of both X-ray crystallography and NMR to identify MET-7 and HIS-106 as the two ligands of the haem iron of cytochrome b_{562} .

Cytochrome b_{562} is a cytoplasmic component of *Escherichia coli*. It contains 110 amino acids of known sequence³ and a non-covalently bound haem group⁴. The sequence is shown in Fig. 1. The molecule contains two histidines at positions 66 and 106 and three methionines at positions 7, 35 and 61. The identity of the haem ligands was previously unknown, although spectral similarities to the *b*-type cytochromes and sequence homology to myoglobin suggested that they were histidine⁵.



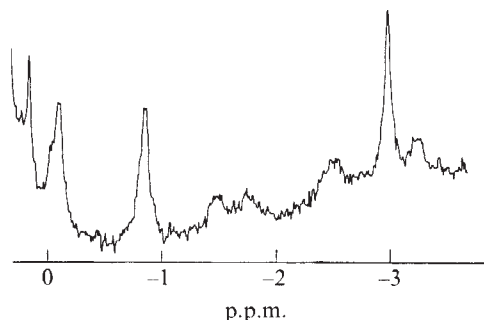
Fig. 1 Amino acid sequence of cytochrome b_{562} (ref. 3). Haem ligands, MET-7 and HIS-106 are circled. The other histidine and two remaining methionine side chains are underlined.

We have been studying the structure of cytochrome b_{562} using X-ray methods. The triclinic crystals contain two molecules in a unit cell of dimensions: $a = 33.71 \text{ \AA}$; $b = 50.48 \text{ \AA}$; $c = 32.73 \text{ \AA}$; $\alpha = 102.76^\circ$; $\beta = 86.55^\circ$; $\gamma = 106.70^\circ$ (ref. 6). The two molecules are related by a non-crystallographic twofold axis nearly perpendicular to the b axis⁷. We have obtained a single uranyl acetate derivative containing one major binding site and four minor sites per unit cell. An electron density map has been calculated at $2.5 \text{ \AA}$ resolution based on X-ray measurements including anomalous scattering from native and derivative crystals. Details of the data collection procedure, derivative preparation, heavy atom refinement and map calculation will be published elsewhere.

The map was averaged about the non-crystallographic twofold axis and plotted on a mini-map at a scale of $1.0 \text{ cm \AA}^{-1}$. The haem group was clearly seen and portions of the polypeptide chain could be traced. However, the density merged at several points, making it difficult to trace the chain unambiguously. The map was then transferred to a Richards box at a scale of $2 \text{ cm \AA}^{-1}$ in order to build a Kendrew model into the density.

It was relatively easy to identify the ligand on one face of the haem group as HIS-106 and to fit its side chain and those of its

Fig. 2 High field region of the 270 MHz ^1H -NMR spectrum of ferrocytochrome b_{562} (0.2 ml 1.5 mM in D_2O , nominal pH = 7.0, 40°C). The spectrum was obtained in a Bruker 270 MHz spectrometer. The solvent resonance was suppressed^{19,20} and 7,000 transients were accumulated in a Nicolett 1180 computer. The sample was reduced with minimal amounts of crystalline sodium dithionite under argon. Dioxan was used as internal standard but all the chemical shifts are quoted in parts per million (p.p.m.) from 2,2-dimethyl-2-silapentane-5-sulphonate.



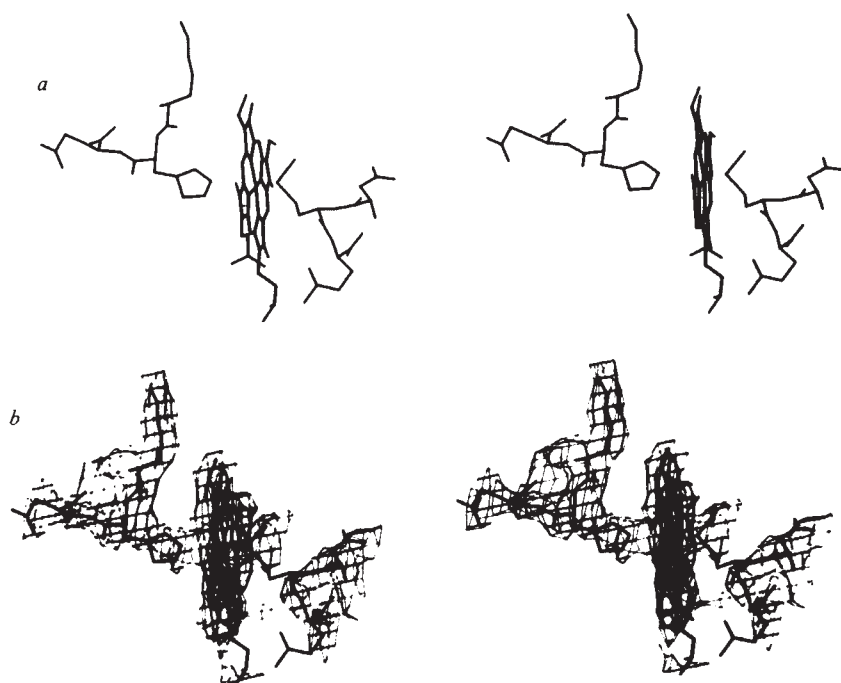


Fig. 3 Stereo photographs showing the haem group and the two ligands with adjacent side chains. The sequence asp⁶-met⁷-gln⁸ is on the right side with gln⁸ at the bottom. The sequence lys¹⁰⁵-his¹⁰⁶-gln¹⁰⁷ is on the left with lys¹⁰⁵ at the top. *a*, Skeletal model only; *b*, skeletal model superimposed on the electron density. The MMS-X molecular graphics computer developed at Washington University was used to produce the photograph. This system was not yet available during the model building stages described in the text.

immediate neighbours into the density. On the other face of the haem group, however, it was not possible to identify the side-chain ligand, and either histidine or methionine (or indeed some other side chain) could be placed in the density.

We decided that an independent method was needed to confirm the fifth ligand and to identify the sixth ligand before the model building could proceed. NMR is a non-destructive method particularly suited for this type of study. A considerable number of cytochromes in which the iron is ligated with either one histidine and one methionine⁸ or two histidines⁹⁻¹² have been characterised using NMR. The chemical shifts of the proton NMR spectrum of a methionine residue ligated to the low spin ferrous ion of a *c* type haem give a very characteristic pattern^{13,14}. These chemical shifts have been used to identify methionine as the sixth ligand of several cytochromes^{15,16}. The electron proton resonance (EPR) spectra¹⁷ of cytochromes *c*₃ from *Desulphovibrio* sp. and the absence of such chemical shifts in the NMR spectra of the low spin ferrous form of these cytochromes¹⁰⁻¹² prove that both the fifth and the sixth iron ligands are histidines. Similarly, the NMR spectra of low spin ferrocycytochrome *b*₅, which has two histidine ligands¹⁸, do not show the typical features of a methionine residue bound to the haem iron⁹.

Figure 2 shows the high field region of the high resolution proton NMR spectrum of reduced cytochrome *b*₅₆₂. The three protons intensity singlet at -2.98 p.p.m. is unequivocally assigned to the methyl group of a methionine coordinated to the haem iron^{13,14}. The one proton intensity resonances at -1.46 p.p.m., -1.75 p.p.m., -2.48 p.p.m. and -3.22 p.p.m. are assigned to the β - and γ -methylene protons of this residue. The one proton intensity singlet at 0.11 p.p.m. is in the region expected for one of the protons of histidine residues bound to low spin haem iron^{15,16} and supports earlier evidence⁵ for the nature of the fifth ligand (HIS-106).

Once it was known that the second haem ligand was methionine, it was relatively easy to identify which of the three it was. Short sequences of polypeptide chain corresponding to each methionine and two residues on either side were built. Each was tested in the density, and one sequence containing MET-7 fit the density much better than the others. The chain was then built into the density from MET-7 toward the N-terminus and forward to about residue 25. The conformation of the chain in this region consists of about four turns of α -helix. Figure 3 shows the model of the haems MET-7 and HIS-106 and their adjacent residues superimposed on the electron density map.

The model of cytochrome *b*₅₆₂ has been completed, but there are still regions of possible ambiguity in the interpretation. The electron density map is presently being refined by the method of molecular replacement²¹. The results and complete structure will be reported elsewhere.

Cytochrome *b*₅₆₂ is the first *b*-type cytochrome shown to contain both histidine and methionine as haem ligands. In contrast to cytochrome *c* the methionine occurs before histidine in sequence. However, cytochrome *c*₃ has *c*-type haems ligated by two histidines. These unusual situations suggest that a more accurate classification of cytochromes may be necessary. The redox potential of cytochromes with two axial histidine ligands is usually lower than that of cytochromes with one histidine and one methionine axial ligand¹². The redox potential of cytochrome *b*₅ ($E'_0 = 20$ mV)²² is lower than that of cytochrome *b*₅₆₂ ($E'_0 = 110$ mV)⁴. The completion of the cytochrome *b*₅₆₂ structure should be important for a better understanding of the structural control of the redox potentials of cytochromes, and of their evolutionary relationship.

This work has been supported by the US PHS and the NSF.

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Received 22 May; accepted 30 June 1978.

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Dynamic model of globular protein conformations based on NMR studies in solution

BASIC PANCREATIC TRYPSIN INHIBITOR (BPTI) is a small globular protein of molecular weight 6,500 (refs 1–17). It consists of one polypeptide chain with 58 amino acid residues, including three disulphide bonds and four phenylalanines and four tyrosines as the only aromatics¹⁸. The crystal structure at 1.5 Å resolution has been extensively refined¹⁹. In the ¹H- and ¹³C-NMR (nuclear magnetic resonance) spectra, a large number of resolved resonance lines have been assigned to particular residues in the amino acid sequence^{6,8,10–16} and related to particular aspects of the molecular conformation^{1–17}. Hence, NMR spectral similarities and differences between BPTI and related proteins can on the basis of these earlier studies be interpreted in terms of structural features in well defined locations of the protein molecule. We report here the use of NMR observations to describe the globular BPTI conformation in terms of a dynamic ensemble of rapidly interconverting molecular structures. BPTI was a suitable compound for this study as its globular form is outstandingly stable in aqueous solution (Table 1)^{1,9,20}, leaving a large experimentally accessible range for studies of modified BPTI species with reduced stability. The chemically modified and homologous proteins used are characterised in the footnotes to Table 1.

Using the extensively refined X-ray structure¹⁹ and the well resolved NMR spectra it has been demonstrated that the average molecular conformations of BPTI in crystal form and in solution are, with the exception of localised regions on the protein surface^{8,17}, very similar^{1,2,14,16} and the structure type

seen in BPTI crystals prevailed also in aqueous solutions of all the proteins in Table 1^{13,21–23}. In this study the information on the solution conformations obtained from comparison with the crystal structure of BPTI was complemented by NMR measurements of the denaturation temperature, the amide proton exchange rates with the solvent in D₂O solution and the intramolecular mobility of the aromatic rings. The conclusions on the molecular dynamics are based largely on comparison of these features in the different structurally closely related proteins of Table 1.

The denaturation temperatures (see Table 1) were determined from the transition of the ¹H-NMR spectrum of the globular proteins to a random coil spectrum^{1,27}. An additional important observation was that at the denaturation temperature, the exchange between globular and denatured protein, was slow on the NMR time scale¹⁴.

The amide proton exchange rates were obtained for numerous individual protons by measurements of the decrease of the resonance intensities with time after dissolving the proteins in D₂O (ref. 27). Approximately 25 interior amide protons could readily be studied using this technique^{1,2}. Table 1 shows rate constants for the most slowly exchanging amide proton in the different proteins^{13,22,23}.

High resolution ¹H-NMR spectra provided an unambiguous criterion to determine rotational motions of the aromatic rings of phenylalanine and tyrosine about the C_β–C_γ single bond²⁷. In BPTI, three aromatic rings were found to undergo 180° flip motions about the C_β–C_γ bond²⁸ with frequencies of the order of 50–1,500 s^{–1} at 40° and activation energies ΔG* of the order of 15 kcal mol^{–1}. One ring was immobilised over the entire accessible temperature range, with ΔG* ≈ 20 kcal mol^{–1} at 80° and four rings were flipping too rapidly to be studied quantitatively^{3,5,9}. In Table 1 the flipping motions for two selected rings are characterised for the different proteins.

Three features of the data obtained are worthy of attention. (1) The amide proton exchange rates are outstandingly slow for globular proteins^{29,30}, with life times of the order of several years for the most slowly exchanging protons in BPTI at neutral pD and 25 °C (refs 1, 2). On the other hand, local flexibility of the protein structures is implied by the rotational motions of the aromatic rings^{3,9,27,28,31}. (2) The amide proton exchange rates are correlated with the thermal stability of the globular conformation, that is, in proteins with lower denaturation tempera-

Table 1 Denaturation temperatures, amide proton exchange rates and intramolecular mobility of aromatic rings in BPTI and six related proteins

Protein*	Denaturation† T _D (°C)	NH exchange‡ k _{EX} (min ^{–1})	Mobility of aromatic rings§			
			Tyr 35		Phe 45	
			ν (s ^{–1})	ΔG* (kcal mol ^{–1})	ν (s ^{–1})	ΔG* (kcal mol ^{–1})
BPTI	>95	2 × 10 ^{–7}	6	16.5	30	14.2
CTI	70	6 × 10 ^{–5}	~6	~16.5	~300	~13.0
HPI	60	5 × 10 ^{–3}	6	16.5	150	13.3
R-BPTI	76	1 × 10 ^{–4}	~6	~16.5	30	14.2
BPTI ¹	88	5 × 10 ^{–5}	—	—	60	13.9
Des(A, R)BPTI	>65	7 × 10 ^{–5}	—	—	60	13.9
TRAM-BPTI	>95¶	—	6	16.5	30	14.2

* BPTI, basic pancreatic trypsin inhibitor; HPI, isoinhibitor K from *Helix pomatia*²⁴; CTI, cow colostrum trypsin inhibitor²⁵; R-BPTI, modified BPTI obtained by reduction of the disulphide bond 14–38, with the cysteinyl residues protected by carboxamidomethylation²³; BPTI¹, modified BPTI obtained by cleavage of the peptide bond Lys 15–Ala 16 (ref. 26); Des(A, R)BPTI, modified BPTI obtained by cleavage of the peptide bond Lys 15–Ala 16 and removal of Ala 16 and Arg 17 (ref. 26); TRAM-BPTI, modified BPTI obtained by transamination of the α-amino group of Arg 1 (ref. 17).

† T_D, temperature at which at pD 5.0 equal concentrations of globular and denatured protein were observed.

‡ k_{EX}, Rate constant for the most slowly exchanging amide proton at pD 4.5 and 36 °C.

§ ν, Frequency at which the rings undergo 180° flips about the C_β–C_γ bond^{5,28}, ΔG*, activation energy for these rotational motions. The data for Tyr 35 were obtained at 27°, those for Phe 45 at 4 °C.

|| In these proteins, the modifications are immediately adjacent to Tyr 35; as a consequence, the Tyr 35 spectrum corresponds to the situation of rapid exchange over the entire accessible temperature range.

¶ BPTI and TRAM-BPTI were compared in different conditions. In 1M guanidinium chloride solution at pH 6.0, T_D was 75° for BPTI and 68° for TRAM-BPTI. At pH 6.0 and 55°, k_{EX} was approximately eight times larger for TRAM-BPTI than for native BPTI.

ture the amide proton exchange is faster. (3) The rotational motions of the aromatic rings are not correlated with the thermal stability or the amide proton exchange, that is, for corresponding rings similar flip frequencies prevailed in the different proteins, unless the immediate ring environment was affected by the protein modification. These observations were used to characterise the globular solution conformations of the proteins studied.

Earlier investigations of amide proton exchange in globular proteins have resulted in the suggestion that the exchange occurs by admixture of 'open' structures, O(H), to the 'closed' globular form of the molecules, C(H) (refs 29, 30). Depending on the relative rates for the closing of the protein, O(H) → C(H), and the acid/base catalysed exchange of exposed amide protons in the open forms O(H), the overall proton exchange rate k_{EX} may be governed either by an EX₁ or an EX₂ mechanism^{29,30}. For BPTI, an EX₂ mechanism was indicated by the pH dependence of k_{EX} in the range 0.5–11.0. The correlation between denaturation temperature and amide proton exchange rates for the different proteins in Table 1 further implies that the equilibrium C(H) ⇌ O(H) involves global variations of the protein structure. This is also supported by the observation that, with few exceptions, the order of the relative exchange rates for the individual amide protons was the same in all the proteins studied^{1,13,17,22,23}. On the other hand, the lack of a correlation between the frequencies of the 180° flips of the aromatic rings and the denaturation temperature or the amide proton exchange rates shows that the open forms O(H), which mediate the exchange of interior amide protons, cannot be in the same class as the thermally denatured proteins. This implies that the local environment of the aromatic rings in the closed form C(H) of the proteins is also essentially preserved in the open forms O(H). Independently, admixture among the open species O(H) of molecular structures corresponding to the thermally denatured protein was excluded on the grounds that the transition from denatured to globular BPTI was slow on the NMR time scale²⁷ even at the denaturation temperature T_D (ref. 14), as a slow closing rate O(H) → C(H) would not be compatible with the observed kinetics of the amide proton exchange¹³.

From the above considerations it seems that the 'globular solution conformations' of the proteins in Table 1, and probably proteins in general, are best described as dynamic ensembles of all the structural species C(H) and O(H) involved in the amide proton exchange, where the Boltzmann law favours population of species closely related to C(H) over the population of more extremely opened forms. The data suggest that previously quoted (refs 30, 32) near-equality in several cases of ΔG^0 between the closed and open protein structures involved in amide proton exchange, C(H) and O(H), and ΔG^0 between the globular conformation and the denatured protein is coincidental. In the proteins of Table 1, structural species corresponding to the thermally denatured forms are excluded by a sizeable energy barrier from the rapid conformational equilibria which govern the exchange of interior labile protons. That the hydrophobic pockets which enclose the aromatic rings in the proteins used are apparently preserved in the open structures O(H) is compatible with the assumption that the molecular fluctuations promoting the amide proton exchange consist primarily of intramolecular translational and rotational motions of intact hydrophobic domains relative to each other. Such fluctuations would primarily open the hydrogen bonded secondary structures which link the different hydrophobic pockets¹⁹. Independently, the mobility of the aromatic rings would be correlated with the internal structural flexibility of the individual hydrophobic domains. Hydrophobic stability domains as evidenced here in one class of globular proteins might quite generally play an essential role in the architecture of protein molecules, a role which may so far have been assigned too one-sidedly to the regular secondary structures adopted by the polypeptide backbone³³. In particular, the lack of correlation of the preservation of the hydrophobic pockets surrounding the aromatic rings with the overall stability of the globular protein conformation

indicates that such hydrophobic domains might be formed at an early stage of polypeptide folding, possibly even as primary nucleation sites for the folding process.

The observed correlation between amide proton exchange rates and denaturation temperature (Table 1) may in this model be rationalised by the assumption that the energy barrier ΔG^* separating the globular conformation from the denatured forms of the protein can be overcome only by cooperative structural fluctuations resulting from overlap of different open structures O(H). With the increase of the population of states O(H) when approaching the denaturation temperature, the probability of such cooperative processes is greatly enhanced. The cooperative fluctuations would also cause destabilisation of the hydrophobic domains, thus causing a sharp transition to the denatured form.

While a thorough comparison of our results with other studies of protein flexibility must be deferred to a subsequent, more detailed paper³⁶, it seems important to point out that the many-parameter characterisation of internal motions in globular proteins by high resolution NMR provides a considerably more detailed description of interior parts of the molecules than that implied, for example, by the terms 'fluid-like' or 'continuous visco-elastic'³⁵ suggested in interpretations of theoretical model studies.

Financial support by the Swiss NSF (project 3.0040.76) is gratefully acknowledged.

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Two contiguous conformations in a nucleic acid duplex

DNA CONFORMATIONS and properties depend on DNA sequence and on environmental conditions¹⁻³. It has been suggested that specific regulatory sites on DNA might have unique secondary conformations². Conformational transitions, and the propagation of these transitions along a DNA helix, have been proposed as mechanistic steps in RNA transcription⁴ and DNA replication^{3,5} as well as having possible regulatory roles in these functions⁶. Whether two significantly different conformations can coexist in a DNA duplex, however, has been uncertain. The *A* and *B* structural forms of DNA differ greatly in base-pair tilt, base-pair positioning and sugar ring pucker^{7,8}. Most DNA duplexes are generally considered to adopt the *B* form in solution whereas RNA duplexes and DNA·RNA hybrids have only been found to adopt *A* forms⁹⁻¹¹. We have synthesised a polynucleotide complex (Fig. 1) which has covalently linked DNA·DNA duplex and RNA·DNA hybrid tracts. NMR studies on this molecule demonstrate that the *A* and *B* conformations can coexist along a nucleic acid duplex and also that significant propagation of conformational transitions along DNA helices is unlikely.

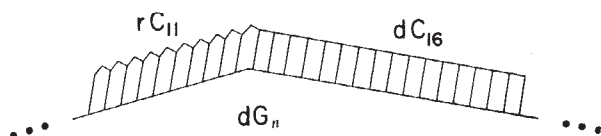


Fig. 1 A schematic diagram illustrating the coexistence of two different helical conformations in the $dG_n \cdot rC_{11}dC_{16}$ duplex.

Synthesis of the block complex, $dG_n \cdot rC_{11}dC_{16}$, was achieved as follows. rC_n and dC_n were hydrolysed to oligonucleotides by partial alkaline¹² and pancreatic DNase¹³ digestion, respectively. rC_{11} and dC_{16} oligomers were isolated from the digests using reversed-phase ion-exchange chromatography (RPC-5) (ref. 14). Alkaline phosphatase treatment removed the 3'-terminal phosphate from rC_{11} ; dC_{16} was acetylated at the terminal 3'-OH position using acetic anhydride¹⁵. rC_{11} and $dC_{16}OAc$ were mixed with dI_n and T4 DNA ligase was used to covalently link the RNA and DNA oligomers¹⁶. The product oligonucleotide, $rC_{11}dC_{16}OAc$, was isolated from both the dI_n template and the unreacted rC_{11} and $dC_{16}OAc$ substrates by RPC-5 chromatography at 65 °C. Finally, $rC_{11}dC_{16}OAc$ and dG_n ($S'_{20,w} \approx 3$) were annealed to form Watson-Crick duplexes according to a previously published procedure¹⁷, which also removed the acetyl group from the 3'-OH terminus of the block oligonucleotide. Four milligrams of $dG_n \cdot rC_{11}dC_{16}$ were prepared for high resolution proton magnetic resonance studies. Physical, spectroscopic and biochemical determinations similar to those reported previously^{2,12-14} were used to characterise this nucleic acid (E.S. and R.D.W., in preparation).

In Fig. 2 the resonances in the proton NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ are compared to control $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$ spectra. For $dG_n \cdot rC_{22}$, the resonance from the hydrogen-bonded G-N₁ proton is at 12.7 p.p.m. and the G-H₈ and C-H₆ protons resonate at 7.2 and 7.8 p.p.m. respectively. The positions of all these resonances are similar to the analogous resonances for $rG_n \cdot rC_n$ (see Fig. 2 legend). X-ray diffraction studies on $rG_n \cdot rC_n$ ⁴ and $dG_n \cdot rC_n$ (E.S., R.D.W., A.G.W. Leslie and S. Arnott, unpublished) in polynucleotide fibres indicate that these duplexes adopt only *A* conformations.

For $dG_n \cdot dC_{22}$, on the other hand, the G-N₁ proton resonance is at 12.98 p.p.m. and both G-H₈ and C-H₆ protons resonate at 7.55 p.p.m. X-ray fibre diffraction indicates that $dG_n \cdot dC_n$ can adopt either *A* or *B* conformations¹⁸. In the conditions of high relative humidity usually extrapolated to solution studies, the *B* conformation is observed. The NMR spectrum of $dG_n \cdot dC_{22}$ supports this extrapolation since the

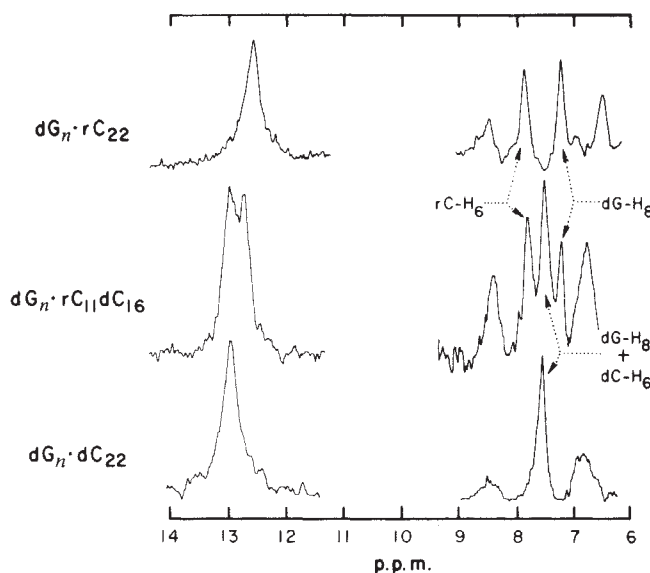


Fig. 2 A comparison of the 300 MHz NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ with the spectra of appropriate control samples. The assignments of the various resonances are indicated. Spectra were obtained using a Varian HR-300 spectrometer operated in the field sweep mode. The spectra shown were measured at 35 °C except for $dG_n \cdot rC_{22}$ which was measured at 22 °C. The low field spectra (12–14 p.p.m.) for $dG_n \cdot rC_{22}$ and for $dG_n \cdot rC_{11}dC_{16}$ are at twice the gain of the spectra for the aromatic regions (6–9 p.p.m.). For $dG_n \cdot dC_{22}$, the low field spectrum is at four times the gain of the aromatic region. NMR samples were prepared by dialysing against a dilute buffer solution and then concentrating the sample 20-fold by rotary evaporation. The final buffer concentration was about 50 mM phosphate, 0.5 mM EDTA, pH 7.0. 2–3 mg of nucleic acid was present in the final volume of 0.12 ml. For $rG_n \cdot rC_n$ at room temperature, the resonance from the hydrogen-bonded G-N₁ proton is at 12.6 p.p.m. whereas the G-H₈ and C-H₆ protons resonate at 7.27 and 7.95 p.p.m., respectively (D. Lerner, unpublished).

observed resonance positions for this complex are consistent with those calculated using ring current shift theory for stacked G·C base pairs in the *B*-DNA conformation^{19,20}. The assignment of *A* and *B* conformations in solution for $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$, respectively, is in accordance with other NMR studies on oligonucleotides which show that ribonucleic acids prefer *A* forms²¹⁻²³ whereas deoxyribonucleic acids prefer *B* forms²⁴⁻²⁶.

The proton NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ shown in Fig. 2 is clearly a composite of the $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$ spectra. The ratios of the resonance intensities at the various positions are consistent with the 11:16 hybrid to DNA duplex ratio of the complex. The obvious conclusion from this spectrum is that the two ends of the block complex adopt two different conformations. Apparently, the significant differences between the *A* conformation of the RNA·DNA hybrid end of the molecule and the *B* conformation of the DNA·DNA end can be accommodated so that the two structures coexist within a single short oligonucleotide duplex.

We recognise that the NMR spectrum presented in Fig. 2 for $dG_n \cdot rC_{11}dC_{16}$ could be interpreted as an equilibrium mixture of duplexes totally in one conformation or the other, rather than two conformations (*A* and *B*) present in a single duplex. This is unlikely since: (1) RNA·DNA hybrids have never been found to adopt *B* conformations; (2) judging from the line widths, the rate of conversion between the two conformations in equilibrium would have to be extremely slow (less than 30 s⁻¹); (3) the equilibrium constant of the system would have to be accidentally very close to 11/16 and (4) when actinomycin D, which binds to DNA duplexes but not to DNA·RNA hybrids²⁷ was added to the block polymer sample, resonances from 12.98 p.p.m. (*B* form) were selectively shifted upfield to 11.9 p.p.m. with no

effect on the intensity in the 12.7 p.p.m. (*A* form) peak (data not shown). This result is difficult to explain in terms of an equilibrium model. We feel these arguments amply justify our straightforward interpretation of the spectra.

The peak positions of the spectra shown in Fig. 2 for the block polymer $dG_n \cdot rC_{11}dC_{16}$ are indistinguishable from the spectra found with 3-component helix $dG_n \cdot (rC_{18} + dC_{18})$ (data not shown). Thus, any disruption of the nucleic acid helix of $dG_n \cdot rC_{11}dC_{16}$, which is created at the junction of the *A* and *B* conformations, is minimal as measured by proton NMR.

X-ray diffraction and infrared dichroism studies have indicated that $dG_n \cdot dC_n$ helices show a greater disposition to adopt *A* conformations than DNAs having random base sequences^{18,28}. Nevertheless, the $dG \cdot dC$ end of $dG_n \cdot rC_{11}dC_{16}$ maintains a *B* conformation despite the adjacent *A* conformation of the RNA · DNA hybrid. This finding provides clear evidence against significant propagation of secondary conformations along DNA helices, and is consistent with earlier NMR studies on $(dC_{15}dA_{15}) \cdot (dT_{15}dG_{15})$ ²⁰ and with investigations of drug binding to DNA²⁹. Propagation of these types of structural transitions in linear DNA cannot, therefore, explain either long-range genetic regulation such as seen in the arabinose operon³⁰ or some of the steps in DNA replication^{3,5}.

The presence of two conformations in a single duplex must necessitate some perturbation of the helix at the junction of the conformations. The peaks in the NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ are not sufficiently resolved to provide detailed information concerning this point. Model building, however, shows that neighbouring *A* and *B* conformations in a nucleic acid duplex can result in a 'bend', that is, a change in the direction of the duplex helix axis, with a junction involving only one base-pair (E.S., R.D.W., C. J. Alden and S. Arnott, manuscript in preparation).

This study demonstrates that two quite different conformations can exist as neighbours on a DNA helix. Thus, sequence-specific conformational sites, possibly present at genomic regulatory regions, could exist if these sequences were approximately one turn of helix (10 base pairs) in length. Shorter conformationally unique sequences might also be allowed. Prime candidates for structurally unique sites on natural DNAs would be the *dA : dT* sequences found in several eukaryotes^{31,32}. $dA_n \cdot dT_n$ has only been observed in the *B'* conformation, a variant of the classical *B*-DNA structure, and never in an *A* form³³. This behaviour may be relevant to the postulated role of *dA · dT* tracts in termination of RNA transcription³⁴ since RNA · DNA hybrids are apparently limited to *A* conformations. Other candidates for conformationally unique sites in DNA include regulatory control regions, for example the *lac* operator has been implicated to be structurally unique³⁵. These concepts have been recently reviewed¹.

This work was supported by the NSF and NIH.

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Received 25 April, accepted 10 July 1978.

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Comparison of theory to experiment for DNA thermal denaturation

THEORETICAL profiles of the thermal denaturation of random sequence DNA that were calculated by Frank-Kamenetskii and Vologodskii¹ displayed a number of narrow subtransitions. In several ways these subtransitions resembled the thermalities observed experimentally in derivative denaturation profiles^{2–7}. Lyubchenko *et al.*⁸ extended their theoretical work to include the known sequence of Φ X174 DNA and claim that not only the shape of prominent subtransitions, but also the number and denaturation temperatures are essentially the same for both calculated and observed denaturation profiles. We suggest that the agreement between current theory and experiment is not as satisfactory as has been implied, and that at least one critical parameter has been neglected in the theory and ignored in the discussions of Lyubchenko *et al.*⁸.

Thermal denaturation profiles were calculated for the double-stranded DNA of Φ X174, using the complete nucleotide sequence published by Sanger *et al.*⁹. These calculations utilised the Fixman and Freire¹⁰ modification of Poland's method¹¹, and assumed the generally acceptable values of parameters chosen by Lyubchenko *et al.*⁸ for their calculations with the same DNA sequence. As expected, our theoretical results for the whole virus DNA closely parallel those of Lyubchenko *et al.*⁸. However, we find that the theoretical denaturation profile of the *HapII*, Y1 restriction fragment of Φ X significantly departs from that published by Lyubchenko *et al.*⁸, rendering the comparison of the calculated profile to the corresponding experimental data⁴ notably less precise. We do not understand this discrepancy.

The theoretical denaturation profiles for the whole virus DNA are illustrated in Fig. 1*a* and *b*, with values of the parameters T_{AT} and T_{GC} (the average melting temperatures of the *A · T* and *G · C* base pairs within a duplex polymer), corresponding to 30 mM and 195 mM sodium ion concentrations, respectively. As noted in Fig. 1 legend, the parameters σ , α , and U are identical to those of Lyubchenko *et al.*⁸; the values for T_{AT} and T_{GC} are based on the values of Lyubchenko *et al.*⁸ (which correspond to 0.1 SSC or 19.5 mM Na^+), together with an extrapolation of $15.7^\circ C / \log [Na^+]$, which brings the midpoint of the theoretical and experimental profiles into maximum correspondence for this DNA.

Evidently, the main features of the theoretical profiles do not vary appreciably with salt concentration, except for the requisite

translation in temperature. Indeed, the small difference in shape between our theoretical profiles is largely removed if the variation of the denaturation enthalpy with salt concentration¹² is taken into account. As previously reported^{7,8}, the main features of the theoretical profiles are insensitive to moderate changes in the parameters σ and $T_{GC} - T_{AT}$, and only small changes in the profile were demonstrable when different sequence termini were used to initiate the calculation. We have also subjected this DNA sequence to a 3% random substitution of base pairs to estimate the influence of possible errors in the sequence of Sanger *et al.*⁹; no visible change in the profile resulted.

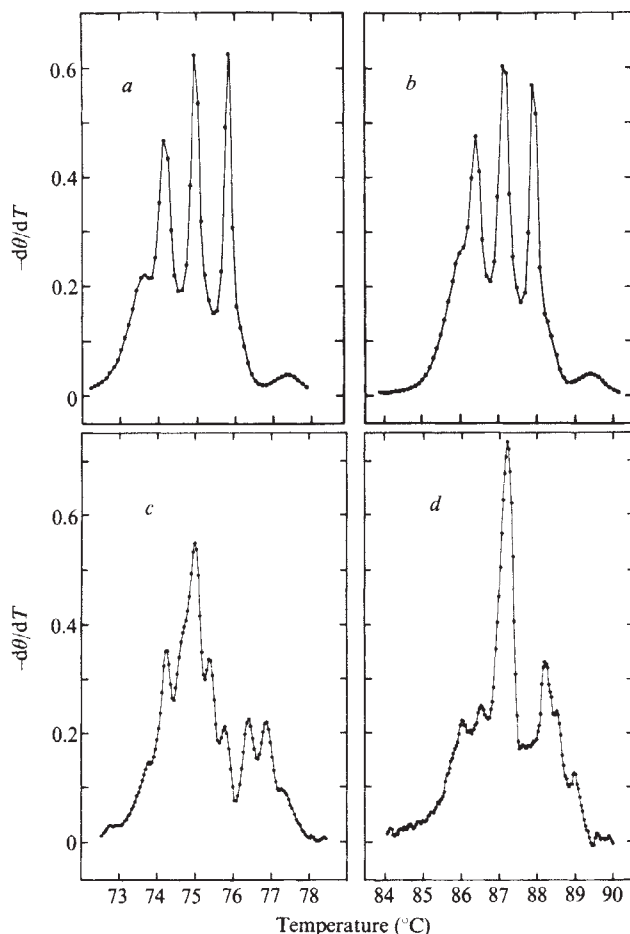


Fig. 1 *a, b*, The theoretical helix-to-coil transition profiles of duplex Φ X174 DNA. The parameters used for this calculation were $\sigma = 5 \times 10^{-5}$ (cooperativity factor), $\alpha = 3/2$ (loop entropy exponent), and $U = 8$ kcal per mol base pair (transition enthalpy). The thermal derivative of the mass fraction in coil form ($-d\theta/dT$) is plotted in (*a*) for $T_{AT} = 328.9$ K and $T_{GC} = 371.3$ K, which should correspond to 30 mM Na^+ , and in (*b*) for $T_{AT} = 341.4$ K and $T_{GC} = 383.8$ K, which should correspond to 195 mM Na^+ (SSC). *c, d*, Experimental high resolution thermal denaturation profiles of Φ X174 RF III DNA. The solution for (*c*) contains 30 mM Na^+ (29 mM Cl^- , 1 mM cacodylate, 10^{-4} M EDTA, pH 7), and that for (*d*) contains 195 mM Na^+ (150 mM Cl^- , 15 mM citrate, pH 7.2). The thermal derivative of the denatured mass fraction ($-d\theta/dT$) was calculated from the optical hyperchromicity at 270 nm using a 9-point least-squares cubic polynomial fitting routine^{2,3}. Standard deviations and fitting routine distortions are approximately the size of the illustrated points. Since the replicative form (RF-I) of Φ X174 cannot denature as a normal linear duplex because of its topological constraints, the initial DNA preparation was subjected to a single double-strand break per molecule using the endonuclease from *Pseudomonas* Bal-31 [*Altaromonas espejiana*]. The repurified RF-III molecules were then subjected to denaturation analysis. We assume that the break occurs randomly along the molecule, as is the case for supercoiled SV40 DNA (unpublished data).

For a quantitative comparison of theory and experiment we have collected high resolution thermal denaturation data on double-stranded am3 Φ X174 DNA in solutions containing 30 mM and 195 mM sodium ions (Fig. 1*c, d*, respectively). The techniques and equipment used were previously described^{2,3}. These results were obtained in conditions that closely approximate equilibrium throughout the helix-to-coil transition and should be directly comparable to the theoretical profiles of Fig. 1*a* and *b*.

Clearly, the thermalites of the two theoretical profiles bear no obvious systematic relationship to the experimental profiles. More importantly, the theory totally fails to reflect the radical changes that occur in denaturation profiles upon variation of the concentration of DNA counterion. In agreement with our previous studies², the number and mean breadth of the thermalites composing our experimental profiles appear similar although the two profiles are obviously distinctive. We must also point out that the mean breadth of the resolvable thermalites in the experimental profiles is consistent with our previous measurements² but is significantly narrower than the subtransition breadths published by Wada *et al.*⁴ for two Φ X174 restriction fragments. This difference probably arises because of our higher thermal resolution^{2,3}. A similar examination of component breadth could not be made on the data of Lyubchenko *et al.*⁸, whose experimental resolution was substantially lower. However, there is a distinct similarity between their 0.1 SSC experimental profiles and our 30 mM profile (Fig. 1*c*), and our experimental results in 0.1 SSC (not shown) are in general agreement, although our resolution clearly delineates maxima and minima where inflections appear in the data of Lyubchenko *et al.*⁸. Further, their 74–75 °C component is several times larger than the corresponding region in our results, which might reflect a bacterial DNA contamination mentioned by those authors.

It must be concluded that our understanding of the process of DNA thermal denaturation is incomplete, and it is not clear what modifications of the theory need be made. Including more sequence information as done by Wartell¹³ or distinguishing sequence doublets as proposed by Borer *et al.*¹⁴ may advance the precision of the calculation, but is not likely to provide the major theoretical revision necessary to accommodate the critical dependence of the denaturation profile upon salt concentration. Indeed, the latter observation may be taken as evidence that an electrostatic determinant differentially influences the stability of cooperative sequences, as we have previously suggested². Until the mechanism underlying this effect has been satisfactorily described, it is clear that the theory of DNA denaturation must be considered inadequate.

This study was supported by NIM grant GM-23067 and NCI grant CA-11430. We thank Dr Horace Gray (University of Houston) for RF Φ X174 DNA preparations.

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matters arising

The *Q*-statistic and the diversity of floras

LAMONT ET AL.¹ accept in principal the *Q*-, or quartile, statistic for floral diversity, combining both equitability and species richness, that we proposed^{2,7} and defined as

$$Q = \frac{\frac{1}{2}S}{\log_e R_2 - \log_e R_1}$$

where *S* is the total number of species in the sample. We selected the 25% (*R*₁) and 75% (*R*₂) quantiles, the quartiles, and evaluated the characteristic slope of the cumulative species abundance curve over this range because, from our experience of insect catches³, a considerable proportion of the species may be represented by singletons. We would expect this quartile statistic to be less affected by sample size than that based on the 10% and 90% quantiles proposed by Lamont *et al.*¹. However, we are more concerned about their justification for the statistic and the results thereby obtained.

Although their characteristic slope, like the *Q*-statistic, may be equated with the reciprocal of the slope of the rank-abundance plot (Fig. 1), standard regression methods are not applicable in the way Lamont *et al.* suggest. Because successive points are very highly correlated, sampling errors will be considerably underestimated, and the method breaks down for discrete data, like those quoted for Malaysian rain forest⁴, in which a large proportion of species are represented by one or two individuals. Our own technique is to fit a stabilising model to the species frequency distribution, rather than to the rank-abundance plot, by weighted least squares⁵. The statistic may then be derived from the parameters of the model (for the log-normal $Q = 0.371 S/\sigma$ and for the log-series $Q = \alpha$) and this method will be highly efficient and applicable for both continuous and discrete data.

Lamont *et al.*'s failure to fit a model¹, in particular the log-normal, seems to discourage them. However, comparison of our Fig. 1 with theirs suggests that the fault lies, not in the models but in their fitting procedures. In spite of the floral richness of their site the abundance distribution does not seem unusual. Further, their curve-fitting tests, based on comparison of the residual sums of squares with the percentage points of a χ^2 distribution (their Table 1), are inappropriate because the size of these residuals will depend on the scaling used.

Using the more appropriate techniques now available in the MLP program⁶ we have fitted the log-normal and gamma³ models (the simpler log-series being unsuitable for continuous data¹) to the heathland data they produced. The fit of both models is good, the gamma slightly the better of the two (Table 1), and, while the two models predict different numbers for the rarest and commonest species, the *Q*-statistics for the midrange species are similar and close to the empirical value obtained by evaluating the quartiles *R*₁ and *R*₂ directly from the sample values (Table 2).

Lamont *et al.*¹ state that the diversity of their heathland sample is "as high if not higher than those for the most diverse communities previously recorded, including the richest of tropical rain-forests". But, when all the diversity indices are evaluated for the data quoted by Lamont *et al.* for Malaysian tropical rain forest^{1,4}, they are found to be consistently higher than those for Western Australian heathland by a factor of between two and three (Table 2).

Table 1 Fit of log-normal and gamma models to species frequency data from the Western-Australian heathland site

Importance value class	No. species observed	No. expected log-normal	gamma
<0.05	14	10.0	17.9
0.05–0.1	8	12.2	9.1
0.1–0.2	20	20.0	13.5
0.2–0.5	35	36.5	27.0
0.5–1.0	23	29.8	28.2
1.0–2.0	30	25.8	32.1
2.0–5.0	30	22.7	33.7
5.0–10.0	10	8.8	9.3
>10.0	2	6.2	1.1

Parameter estimates:

log-normal ($\chi^2 = 11.7$, $P > 0.05$)

$\mu = -0.53$, $\sigma = 1.57$

Gamma ($\chi^2 = 8.2$, $P > 0.05$)

$m = 1.51$, $k = 0.60$

The chi-squared goodness of fit test relies on the assumption that number of species observed in each class is a Poisson variable.

Table 2 Values of indices of diversity for Malaysian rain forest and Western Australian heathland

Index	Formula	Tropical rain forest Malaya ⁴	Heathland site Western Australia ¹	Ratio forest/heath
Simpson's (inverse)	$\frac{1}{\sum_{i=1}^s p_i^2}$	84.2	32.7	2.57
Shannon-Wiener (exponent)	$\exp\left(-\sum_{i=1}^s p_i \log p_i\right)$	149.1	69.4	2.15
<i>Q</i> -statistic	$\frac{\frac{1}{2}S}{\log(R_2/R_1)}$	104.6	38.2	2.74
	log-normal	105.9	40.6	2.61
	gamma	105.6	38.6	2.74
	log-series (α)	114.2	28.0*	
Whittaker's	$\frac{S}{4[(\sum_{i=1}^s (\log p_i - \log \bar{p})^2/S)]^{1/2}}$	77.1	28.3	2.72
log-cycle	$\frac{S}{\log p_1 - \log p_s}$	80.7	21.2 (24.4)†	3.81 (3.31)†

All indices given by Lamont *et al.* are included with the exception of Gleason's index whose value is directly related to population density. *S* is the total no. of species in the sample, *p_i* the relative frequency of the *i*th most frequently occurring species, $\log \bar{p} = (\sum \log p_i)/S$, *R*₁ and *R*₂ the quartiles of the frequency distribution. The logarithms are taken to base *e*. The *Q*-statistic is derived directly from the data and from the parameters of three fitted models.

* The log-series diversity index α is not directly comparable with other indices for the heathland site as it is calculated from the species numerical abundances not their IVI importance values.

† Bracketed figures are obtained by omitting single rarest species. This operation has a negligible effect on other indices.

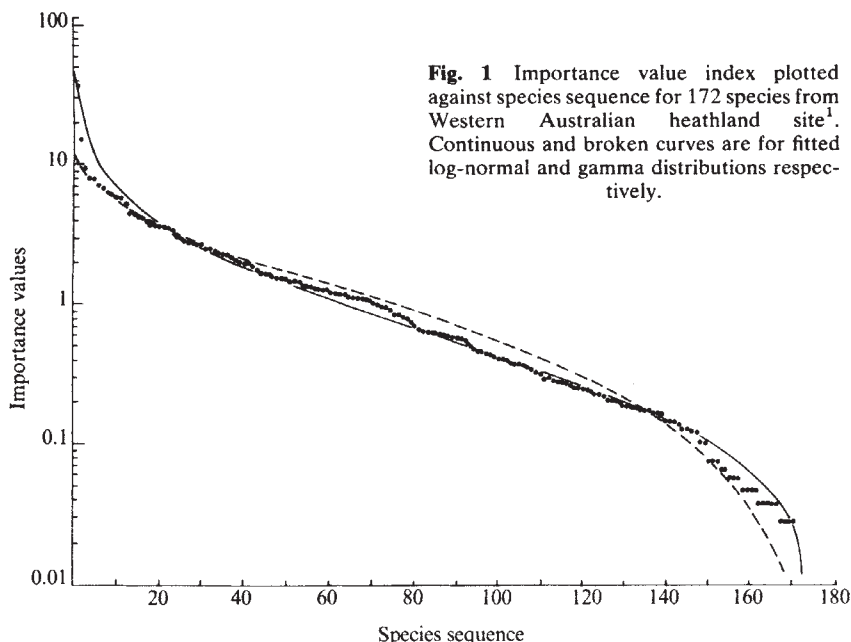


Fig. 1 Importance value index plotted against species sequence for 172 species from Western Australian heathland site¹. Continuous and broken curves are for fitted log-normal and gamma distributions respectively.

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LAMONT ET AL. REPLY—Use of the best-fit ('characteristic') slope of the importance-value curve as a measure of diversity has intuitive appeal. The problem in species rich samples, however, is that nonlinear models generally fit the data better than linear ones. In view of this, use of the variance might prove an acceptable alternative. We maintain that both these measures are indices of evenness and not of total diversity, such as Shannon's which confounds both evenness and richness (Fig. 1).

Comparison of our heathland data against results for Preston's classic curve-fitting procedure for the log-normal distribution^{1,2}, using the χ^2 test favoured by Kempton and Taylor³, confirms our previous conclusion of their dissimilarity⁴. Using the more recently devised methods

of estimating log-normal and gamma parameters, adopted by Kempton and Taylor, we now see that the difference becomes nonsignificant. Because lumping may mask variation in the original data, particularly in the dominant-species region (a limitation of the variance approach to evenness) it is desirable to compare the log-normal curve against the observed curve. In our original paper⁴, the scaling was fixed so the χ^2 values for the fitted curves gave some indication of their relative goodness of fit, but the significance levels should be ignored. We relied, however, on the more appropriate one-sample, Kolmogorov-Smirnov test⁵. The observed and expected importances for the log-normal fit by Kempton and Taylor above were significantly different at the 0.1% level,

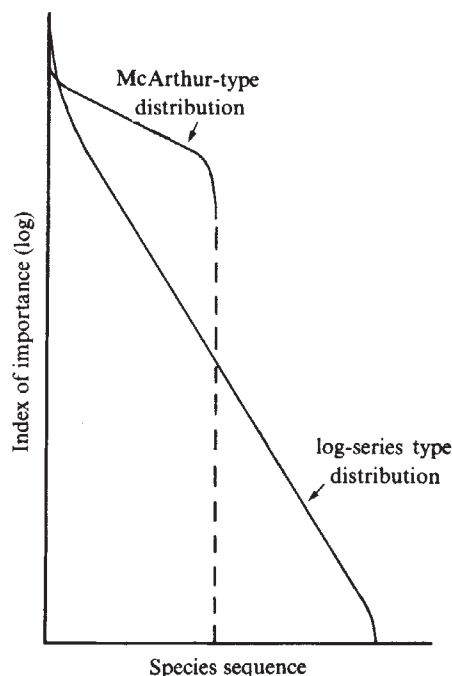


Fig. 1 The relationship between evenness and total diversity: one community (for example representing a McArthur distribution) has half the slope (twice the evenness in terms of number of species per unit importance), but only half the number of species (half the richness) as the other (for example representing a log-series distribution). Only when these figures are multiplied do we have an indication of total diversity—in this case they should be the same.

and for the gamma fit at the 5% level. The latter is worse than the log-series, but better than the (linear) exponential⁴.

We agree with Kempton and Taylor that the gamma index (S/σ^2) is the best variance measure of evenness, even though it does not fit all data and is not synonymous with the best fit slope. It should not be confused with the quartile index which is an inferior slope measure³. The exponential curve remains the best measure of slope, though removal of the dominant species is sometimes required to meet the requirements of the Kolmogorov-Smirnov test, with minimal change in slope. We now consider removal of up to 10% from each tail (our original characteristic slope⁴), let alone 25% (quartile), is unwarranted.

We made it clear⁴ that William's index, based only on number of individuals (largely a function of life form), for the Malaysian rain forest was much higher than for the Western Australian heathland. We are therefore not surprised that data we considered of little relevance to community diversity (except in providing species richness/number of individuals), and incompatible with ours, continues to show a higher diversity when expressed in terms of other indices. In addition, we point out that the rain forest site was not physiographically uniform⁶, it is difficult to identify communities in this rain forest anyway⁷, the method of survey ensured high evenness: specimens had to be tall trees exceeding 30 cm in diameter but no reference was made to their size, and the sample was nearly 450 times the area of ours and 20 times its height. Had small plants been included and more acceptable measures of importance been used it is certain that the vast increase in range of values would have greatly reduced evenness. Such inclusions would no doubt have increased the slope of the species-area curve of the rain forest above that of the heathland, but the extensive experience of one of us in related Dipterocarp rain forests indicates that ten 50 m² samples which contained 150 species, let alone 172, would be most unlikely.

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Renewable energy sources and storage

LEICESTER, NEWMAN AND WRIGHT¹ claim to have invalidated Ryle's² hypothesis that windpower could make a substantial and economical contribution to Britain's electricity supply, once short-term (150-h) thermal storage is provided at places of end-use. The counter-argument of Leicester *et al.*¹ seems to be based on two principal observations. (1) Plots of average heat demand against mean windpower output, which show that the standard deviation of windpower output is large and decreases only slowly with storage capacity. And (2), their "extreme example" for which the mean wind energy available over the period 1 February–31 March 1975 was about 54% of the long-term average wind energy. Unfortunately, neither of these observations provides a test of Ryle's hypothesis.

Observation (1) confirms the well-known fact that windpower is very variable. However, since the probability distribution of windpower is highly skewed in general, it is not particularly useful to quote standard deviations. More importantly, the scatter diagrams do not necessarily provide useful information on the crucial problem of storage content. The proposition by Leicester *et al.* that their interpretation of Ryle's thesis implies that the points in their Fig. 2b should be grouped around the straight line through the origin of slope unity, is false. The grouping is sufficient but not necessary. Because windpower is proportional to the cube of windspeed, the store can be filled quickly but will be emptied slowly.

A simple example brings out this inadequacy of the scatter diagrams. If the daily output is constant at 1 unit, which seems to be a reasonable first approximation to the data presented, then 11 days' successive input of $\frac{1}{2}$ unit followed by a day with an input of 6 units keeps the store non-empty at all times, given that it was initially full. Yet the running 6-day average inputs are $\frac{1}{2}$ for half the time and $1\frac{1}{2}$ for the rest, giving a wide scatter.

In contrast to their scatter diagram (Fig. 2) which is not helpful, quantitative information on windpower reliability is obtained from plots of power availability or capacity factor expressed as a function of storage capacity^{3–5}.

Observation (2) merely reiterates the well-recognised point that windpower plus short-term storage alone cannot satisfy power demand with a probability of unity. However, Ryle² does not assume that there are no conventional sources of power feeding the grid. We suggest that some of the real questions of quantitative significance raised by Ryle's work are as follows. First, what is the probability of low-wind periods of a given length? Second, how much conventional (that is,

non-renewable) baseload, intermediate load and peakload capacity must be retained in a grid fed by windpower to ensure that low-wind periods can be covered with a probability close to unity? Third, what are the additional economic costs and benefits of such a hybrid grid? For example, the costs of extra start-ups of conventional power plants during low wind periods and the losses of unutilised windpower when storages are full; the benefit in replacing new or retired fossil-fuel and nuclear plants with windpower or other renewable sources of energy; fuel-saving benefits when de-controlled as well as controlled prices are considered for fossil fuels. Finally, by how much are the answers to the second and third questions changed when a more flexible method of load switching and pricing is introduced? Such an automatic rationing system is technically feasible and could mean that instead of freezing over the period 7–16 February 1975, CEBG all-electric householders would have experienced simply a fall in home temperatures of (say) 5°C over the longer period 2–16 February. For the case of central or substation storage, we have found that a user strategy in which part of the windpower output contributes to the immediate demand and part is proportional to the fraction of energy in storage, yields a substantial increase in the availability of windpower, compared with the strategy of simply following demand (M.D. and K. Malafant, in preparation).

Leicester *et al.*¹ do not attempt to modify user demand, nor consider probabilities of extreme events, nor hybrid systems, nor economics of any kind. Their interpretation of their scatter diagrams is based on a false proposition. It is, therefore, difficult to understand the basis of their assertion that Ryle is "probably incorrect" on relative economics and that "sufficient work has already been done to demonstrate that 150-h storage is quite inadequate to smooth the output of wind generators".

We thank Jeff Wood, Sue Carpenter and Brian Martin for helpful discussions.

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LEICESTER ET AL. REPLY—Since our earlier work we have extended our analysis to determine the amount of storage that would have been needed to match heating demand to windpower output throughout the period 1 October 1974 to 31 March 1975, assuming an energy

balance over the period as a whole. We have done this by calculating and then adding together the maximum cumulative surplus and deficit of windpower output relative to heating consumption over the 6 months. The storage requirement turns out to be 1,124 h, thus substantiating our contention that 150-h storage is quite insufficient.

We have also calculated the normalised averages of heating demand and windpower output for 1,124-h periods within the 6 months and find that the former extend from 0.9 to 1.04 and the latter from 0.4 to 1.73. The standard deviation of the heating demand is now 0.028 and that of the wind energy is 0.394, which are about half the values with 150-h storage. Although these results support Diesendorf and Westcott's argument that zero scatter is not a necessary condition of sufficiency of storage, they do show that with sufficient storage the scatter is very much smaller than for 150-h storage as shown in our Fig. 2b.

Diesendorf and Westcott raise a number of other questions which, we agree, are relevant in a wider context. However, there is clearly a difference in view between us on what Ryle has proposed. Our understanding of his concept is that, he contends that, by controlling the electrical input to storage for space heating and hot water supply by remote switching in accordance with the availability of windpower output, the heating demand can be fully and reliably satisfied without recourse to supplies from other generating plant. We believe that our analysis shows conclusively that 150-h storage is not sufficient to support Ryle's contention, but this does not mean that the use of storage with renewable energy sources is unimportant. Indeed, while it was not the purpose of our article to do more than examine the storage proposition as formulated by Ryle, we are nevertheless continuing to study the question of integrating large amounts of windpower into the CEBG's system and this will include consideration of questions (1), (2) and (3) posed by Diesendorf and Westcott.

With regard to their fourth question, a policy of selective rationing of electricity for heating is not feasible since consumers could not be denied freedom to resort to electrical supplementary heating at times of storage deficiency in cold spells. Such use would inevitably occur and the consequent peak load on the supply system could be close to what it would have been without windpower generation with the result that the latter would have minimal firm capacity.

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reviews

Understanding speciation

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Modes of Speciation. By M. J. D. White. Pp.455. (Freeman: Reading, 1978.) £18.50.

SPECIATION involves the splitting of a single evolutionary lineage into two or more genetically independent ones. This process of cladogenesis is one of the twin axes of organic evolution, the other being the process of anagenesis which involves the phyletic change of an evolutionary lineage through geological time. In the *Origin of Species by Means of Natural Selection* (1859) Charles Darwin dealt with both of these fundamental processes, although perhaps understandably at that time he was more concerned with incontrovertibly establishing anagenesis ("descent with modifications") than with discussing the mechanisms of cladogenesis ("the origin of species"). Nevertheless in chapter four a few short passages reveal that he considered both disruptive selection to fit distinct niches and geographic isolation on islands as important mechanisms of evolutionary divergence. In fact Alfred Russell Wallace (1901) considered the process of speciation more closely.

The 'Neo-Darwinian Synthesis' of evolutionary theory was made some thirty years ago and continued to emphasise phyletic change. However, geographical isolation was now considered to be of prime importance in speciation and by some as a necessary pre-condition. Consequently, nearly all books on evolution devote only a chapter or so to cladogenesis. This remains so in the recent stimulating text *Evolution* by Dobzhansky, Ayala, Stebbins and Valentine (1977), and Verne Grant's thoughtfully integrated *Organismic Evolution* (1977) devotes one of eight major sections to speciation. Ernst Mayr gives the topic major prominence in his *Populations, Species and Evolution* (1970), but this book considers only animals and like its parent volume *Animal Species and Evolution* (1963) vigorously favours geographical isolation. Grant's *Plant Speciation* (1971) is the only book to deal specifically with cladogenesis, but as the title indicates it is restricted to plants.

Against this background Michael White has decided to produce a "comparative study of speciation in relation to the population structure and genetic

architecture of living organisms". He deals with both animals and plants and considers a wide range of possible modes of speciation. This book may thus be considered as the first comprehensive treatment of the origin of species.

It is also a most timely publication. There has been an accumulation of pertinent studies over the past twenty years, the most striking discovery being the considerable level of genetic variation revealed by gel electrophoresis of proteins. And there have also been substantial advances in the less well publicised fields of chromosome variation and behavioural isolation. Some of these investigations have raised nagging doubts as to whether an extended period of geographical isolation is a necessary prerequisite for the production of genetic isolation and speciation. This growing concern can be sensed in the later texts referred to above and in a number of recent papers and reviews. As White himself recognises, "the time therefore seems ripe for a reconsideration of the whole question of the mechanisms of speciation . . . It is probable that a number of modes of speciation exist, and that the simple antithesis of allopatric and sympatric modes is a false one, since most of these involve a geographic component of some kind".

White admits in the preface that "the organisation of the book presented some major difficulties. Many controversial topics could have been discussed equally well in any of several chapters". Nevertheless, the structure which he has chosen to deal with this immense subject proves a successful vehicle to convey his message. Furthermore, the book is very readable, usefully illustrated and well produced. It contains seventy-six pages of references alone and though most of them are recent, it provides a valuable link with the older literature.

Chapter one is a lively introduction; in it he is at pains to convince doubters that species and speciation are identifiable concepts and worthy of study. This leads to a consideration of isolating mechanisms, the genetic processes involved in evolution and the type of data required for a full study of each case of speciation. He is unhappy with previous classifications of models of

speciation and proposes seven models that span the range from pure allopatry to pure sympatry. These models are then dealt with in detail in later chapters, as are polyploidy and parthenogenesis.

Before this exposition the stage is set by a chapter on the genic structure of species and a chapter on chromosomal differences between species. The former provides a balanced and pertinent account of the mushrooming data on electrophoretically detected allelic differences and the questions raised by this. Perhaps the most important conclusion is that related species may differ very little in their allozymic constitution. The massive chapter on chromosomal differences is reminiscent of the author's encyclopaedic work *Animal Cytology and Evolution* (1973). With such a vast array of information at his command the initial heading of sections certainly aids the reader, and it is a pity that this is discontinued after the section on *Drosophila*. It is his expressed intention to advocate a much larger role for chromosomal rearrangements in speciation than in earlier texts and he certainly hammers home the portentous fact that very few, if any, closely related species are chromosomally identical. From this he concludes that these chromosome changes cause speciation, but does not present fully an argument to justify this. Nevertheless, the association of chromosomal difference with speciation requires an explanation.

The allopatric models of speciation in which genetic isolation is acquired under complete geographical isolation are examined in chapter four. Several clear cases are discussed and a number previously claimed as examples are critically reassessed. These necessarily include the Galapagos Islands, the Hawaiian Drosophilidae, the land snails of the Society Islands and Caribbean and the species flocks of ancient lakes, as well as lesser known work in frogs, locusts and lemmings. Character displacement and reinforcement of species-isolating mechanisms are briefly treated at the end of the chapter; these contested subjects are perhaps worthy of more fundamental consideration.

Clinal and area-effect models are considered next. These propose the segregation of distinct species out of

geographical races that are spatially continuous. The thrust of this chapter depends firstly on Bryan Clarke's (1968) model for which there is suggestive evidence and secondly on the reinforcement of hybrid zones over which White is perhaps prematurely enthusiastic. However, these models are as yet relatively under-explored, and he is right to promote interest in them.

The chromosomal modes of speciation are considered seriously in the sixth chapter to account for the widespread occurrence of karyotypic differences between sister species. Harlan Lewis' pioneer work with *Clarkia* argues powerfully for saltational speciation as a form of microallopatry involving hybrid chromosomal sterility, and the bulk of the chapter describes cases that White considers to support his own stasipatric model. This latter is essentially a clinal model driven by hybrid chromosomal sterility. Some authors will possibly be surprised to find their data so interpreted, particularly when they themselves have invoked geographical isolation. Both this chapter and the last are concerned with what Murray (1972) has called parapatric speciation and further investigation will clarify our concepts.

The chapter on sympatric models of speciation is well written and the concepts are readily grasped. This is a result in part at least of the resurgence of interest in the early 1960's through studies such as those of Maynard-Smith and Thoday and Gibson on disruptive selection. The strength of the case rests with the evidence from host-specific insects, particularly that of Bush (1974) on *Rhagoletis*. White makes a good argument for sympatric speciation on small oceanic islands with many species and also for allochronic speciation.

Following a neat review of speciation by polyploidy, White considers it to be fairly insignificant in most organisms, excepting certain groups of plants such as ferns and grasses where it may perhaps have inhibited speciation by other methods.

Chapter nine extends the species concept to include isolation by asexual reproduction and we are treated to an exposition of one of White's pet subjects. He demonstrates the hybrid origin of many thelytokous species, but one wonders if heterosis of the hybrid is absolutely necessary to explain their origin. In the invertebrates at least thelytoky is a sporadic development and he emphasises that though much has been written about the vast agamic complexes in plants there are very few of these. His consideration of the currently engaging question, "What biological use is sex?" is not complete but certainly points the way, and he advocates examination of the genetic architecture of thelytokous species and

their bisexual relatives as presently more necessary than model building.

I found the last chapter (Conclusions) the most enjoyable. It contains all sorts of evidence, concepts, ideas and questions that arise from the preceding survey. One might argue that some of the points on behaviour, sex, hybridisation and reinforcement should have been considered before the models, but one must break into the cycle of scientific enquiry somewhere and this approach is certainly realistic and stimulating.

A comprehensive study of speciation

Automated chess

The Machine Plays Chess. By A. G. Bell. Pp. 114. (Pergamon: Oxford, New York and Sydney, 1978.) Hardback £5; paperback £3.

It is one of the rarer pleasures of a book reviewer to receive a text which both entertains and informs him simultaneously. This book was the source of one such pleasure.

The text commences with an excellent commentary on the principal stages in the historical development of chess automation, going back through the pre-electronic "paper machines" of the early twentieth century, the ideas of Babbage and Turing, and the famous "Turk" pseudo-automaton of the eighteenth century.

The main part of the book deals with the situation in recent years, and

requires the use of a wide range of biological studies. This text will no doubt provoke specialists in many areas, but there are so few people capable of giving us such a fine prospect. This personal view should be read by every serious biologist, for Michael White has succeeded in producing a most readable, comprehensive and stimulating thrust towards understanding speciation—may it bear fruit.

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succeeds admirably in communicating an insight into the 'thought processes' of computer programs (and their authors). The selection of example games clearly shows the super-human capabilities of the computer in tactical calculation, and with equal clarity exposes the perpetual undercurrent of planlessness which is the trademark of all automata. A sensible appendix supplements the main text with a simple explanation of the special techniques used in move selection and evaluation.

This is a book which can be recommended as almost essential for those interested in machine intelligence, would be entertaining for chessplayers of all standards, and provides an excellent introduction for those with experience of neither.

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Neural mechanisms of memory

Elements of the Behavioural Code. By Francis V. DeFeudis and P.A.F. DeFeudis. Pp. 397. (Academic; New York, London and San Francisco, 1977.) £15; \$29.35.

WHAT progress is being made toward the understanding of neural mechanisms of memory in terms of biochemical and anatomical changes in the brain? This seems to be the overall problem set by DeFeudis and DeFeudis. Their main effort in this book has been devoted to surveying changes observed in the nervous system as a consequence of exposing experimental animals to enriched or impoverished environments, or giving them training, or using physiological and/or pharmacological techniques to alter the functional load imposed on the nervous system. Proceeding generally from broader to finer effects,

the authors describe changes found in the gross anatomy of the nervous system, in cellular and subcellular morphology, in neural macromolecules, in energy metabolism, and in presumed neurotransmitters and some other small molecules. This survey is based on an impressive list of over 1800 references, about three-fifths of them from the 1970s and including many citations from 1976 when the manuscript was completed. Following the 89-page reference list are a 29-page subject index and a 25-page author index. The selection and compilation of this material and rendering it accessible through the detailed indexes are sure to be of great help to investigators and advanced students in all the disciplines that are contributing to the study of the biology of memory.

Given the magnitude of the task, it is understandable that DeFeudis and DeFeudis have been somewhat less successful in describing and interpreting the varied and rapidly accumulating material of this field than they

were in compiling the references. The text itself is rather brief in relation to the apparatus of references and indexes; 248 pages of text, tables and figures could hardly be expected to describe the references cited, let alone discuss them to any degree of thoroughness. Many important studies are given only a few sentences, whereas others are mentioned in a phrase or are lumped with other related papers in an overall characterisation. There is not much critical discussion of experiments or conclusions offered by investigators, although the authors occasionally point out discrepancies among reports and suggest some factors that complicate research and interpretation.

The title and prologue of the book may set up too high expectations by indicating that "... a theory for the 'Behavioral Code' is provided ...". Probably the emphasis should be placed on the word "elements", and perhaps "ingredients" would have been a more appropriate term. Table 80 lists as "Elements of the Behavioral Code" items as diverse as environmental stimulation, initiation of nerve impulses and transmitter release, effects on rates of synthesis of cyclic nucleotides, changes in synaptic efficiency, and

changes in learning, memory, and adaptive behaviour. Elsewhere in the book we are told that both short-term and long-term memory are elements and that "... neurotransmitter suspects [that is, presumed neurotransmitters] are integral elements of the Behavioral Code."

Apparently, although no definition is given, the Code when completed will include all of the events and processes that must intervene between initial capture of sensory information and storage of memory. Even the retrieval of memories should be included, although this book reflects most of the work in the field by not even mentioning retrieval. DeFeudis and DeFeudis believe, as do many workers in this field, that storage of memory requires a complex series of processes rather than depending upon some one key process. Their book is a substantial contribution towards the eventual formulation of a comprehensive theory of the neural mechanisms of learning and memory.

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Vertebrate visual system physiology

Handbook of Sensory Physiology. Vol. VII/5: The Visual System in Vertebrates. Edited by F. Crescitelli. (Springer: Berlin, Heidelberg and New York, 1978.) DM 340; \$149.60

THIS volume contains contributions by Crescitelli, Dvorak, Eder, Granda, Hamasaki, Holmberg, Hughes, Locket, McFarland, Meyer, Muntz, Munz, Olson and Reyer. The subject matter is concerned with the morphology, optics, pigments, physiology and adaptive radiation of the eye in different vertebrate classes. The chapters are comfortably short; most are under one hundred pages in length.

This volume is both necessary and important as a supplement to the earlier volumes in the *Handbook*. It is a necessary text for those libraries which deal with visual science, whether in its medical, biological or behavioural aspects, and is important in providing a source-book which is reasonably comprehensive and broad in outlook. It is occasionally suggested that large texts are on the verge of extinction

because of the rate of publication of new papers, and because they can be compendious rather than critical. The latter comment applies only in part to this volume, where a few sections might have been sign-posted better, and where the literature might have been treated more sceptically. Most research workers in vision could find flaws and omissions where their speciality has been touched on, yet would surely benefit from and be stimulated by the breadth and scope of this book.

Although it is probably unfair to pick out single contributions for comment, for all the chapters are of high quality, I was particularly fascinated by the treatment given by Hughes to comparative optics. This chapter shows how much more there is to retinal organisation in mammals than the cat, and is essentially a modern critical essay in the spirit of Gordon Walls.

I applaud this book, and hope that despite its price, it will be widely appreciated by students and research workers interested in vision.

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Spectroscopic techniques

The Spectrum in Chemistry. By J. E. Crooks. Pp. 313. (Academic: London, New York and San Francisco, 1978). Hardback £12.60; paperback £6.50.

THIS is a straightforward textbook on spectroscopic techniques which are used in modern chemistry. All regions of the electromagnetic spectrum are covered, and the book is stronger than most of its rivals in the areas of X-ray spectroscopy, of photoelectron spectroscopy and of dispersion and dichroism associated with optically active materials. Indeed, chapter 9, which introduces optical activity through helices rather than asymmetrically substituted carbon atoms, is especially clear and a sufficient reason to recommend the work to undergraduate science libraries. The whole is well balanced and should be sufficient in depth for most British undergraduate courses. Thus, selection rules are briefly covered where appropriate but transition moments are not introduced. It is stronger on the general ideas and on spectroscopy in its own right than it is on quantum theory or on applications to organic or inorganic chemistry.

The author uses SI units, which is an attraction, although one wishes he did not so often remind the reader by attaching the units to the symbol for the physical quantity. For example, following equation (1.3), into which numerical values can be inserted using any set of coherent units, he adds:—"k is Boltzmann's constant in J K⁻¹". J K⁻¹ may prove convenient units but they are not essential and k stands for Boltzmann's constant in any units. The book has some 20 questions with answers. It also has its share of blemishes and errors, but these are mostly misprints or details which are wrong and do not detract from the qualitative message; they would be more important to the practitioner than an average student.

It is interesting that, as far as the reviewer can discover, Dr Crooks' personal research interests have been in rapid reactions and in solute transfer with emulsions, so that he is not an established expert in spectroscopy. There is much argument as to whether experts or others are to be preferred as undergraduate textbook writers: this book shows the demerit of the inexperienced with its occasional errors, but also shows the merit of clarity and balance to be sought from 'an outsider'.

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Extranuclear genetics

Extranuclear Genetics. By G. Beale and J. Knowles. Pp. 142. (Edward Arnold: London, 1978.) Paperback £5.95.

Few areas of genetics have grown so rapidly and spread so pervasively as has extranuclear genetics in the short time since it achieved some measure of respectability. Whether one dates this from the discovery of DNA in extranuclear organelles or from the publication of the first textbooks to bring the wealth of the cytogenetical evidence for extranuclear hereditary systems to a wider audience makes no difference to the time-scale. It will, however, probably colour one's reaction to the balance of material and the historical perspective which Beale and Knowles present in their account of the subject.

The bulk of the material on extranuclear genetics *sensu stricta* is contained in three chapters devoted to mitochondria, chloroplasts and miscellaneous, respectively. A further chapter on endosymbionts and viruses provides background information from extranuclear systems which may have evolutionary and certainly have phenomenological affinities with the hereditary systems located in mitochondria and plastids. A fifth chapter on plasmids resists the efforts of the authors to justify its presence. A brief introduction and conclusions complete the contents.

The authors deliberately chose to present a relatively brief resumé of recent work on *Chlamydomonas* because of the availability of up-to-date accounts. Their decision to give even briefer accounts of the extensive work on flowering plants, much of it published in German, is, however, more a reflection of the overall strategy of the presentation, with its main emphasis on the molecular and fine structure and a lesser supporting role for genetic analysis. This emphasis shows up in many ways but probably the clearest is that of the total of 36 figures, 26 of which are electron micrographs; only one is a genetic analysis and that concerns an endosymbiont. There is a corresponding shortage or delayed description of the basic details of the life cycles of key organisms without which the genetical analyses are difficult to follow. For example, it is not until more than half-way through the chapter on mitochondria and after the sections on respiratory mutants and drug resistance that the timing of meiosis in yeast and the haploidy of the ascospores are first mentioned.

But it is not this imbalance but an overcautiousness in interpretation at times bordering on scepticism that concerns me most. The high point is where, with only two possible explanations, we are told that there are almost insuperable objections to both! Personally I find this depressing, others may well applaud it as scholarly. Indeed, *Extranuclear Genetics* presents a view of the subject that many and

perhaps all recent converts and those coming into contact with it for the first time will find palatable and a more than adequate introduction. But I doubt whether many will find it stimulating.

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Energy concepts

The Concept of Energy. By E. J. Hoffman. Pp. 573. (Ann Arbor Science: Michigan, 1978.) £18.60.

LET it be admitted at once that this book does not exactly appeal to this reviewer. But it is clear that the author has specialised knowledge in the field. He has written on extractive distillation (1964) and on energy transformations (1970) and is known in the fuel industry. As a result, his treatment of the "mathematical origins of the concept of energy" seems to strive for a universality including philosophy and differential geometry, which the author cannot attain. The result is an uneven

book with undue elaboration of the Joule-Thomson effect (28 pages) and enthalpies of mixtures (60 pages), and nothing is said about the economic assessment of alternative energy systems, the role of energy in biological systems or the reduction in status suffered by the energy concept at the hands of relativity theory. The references are mainly pre-1962 and there are doubts, which do not represent the scientific consensus, concerning relativity (p446) and the constancy of the Stefan-Boltzmann constant (p432). Nevertheless, the considerable effort made by the author is not wasted as the book may prove to be rewarding reading for those with an interest in the specialised topics mentioned above.

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Phase transitions in solids

Phase Transitions in Solids: An Approach to the Study of the Chemistry and Physics of Solids. By C. N. R. Rao and K. J. Rao. Pp. 330. (McGraw Hill: Maidenhead, UK, and New York, 1978.) £18.15.

ALTHOUGH the subjects treated by Rao and Rao are fascinating, I found myself somewhat disappointed by the book itself. This survey of phase transitions in solids is impressively broad, covering topics such as crystal chemistry, nucleation and spinoidal decomposition, soft modes, and the modern theory of phase transitions. Perhaps as a result, the treatment of many topics is frustratingly incomplete and sketchy. The three paragraph discussion of the Peierls transition in one-dimensional systems on pp 294-5, for example, is of little value to the uninitiated. Plots of the conductivity and resistance of KCP and TTF-TCNQ are shown, without a clear explanation of what is supposed to be going on. The authors refer rather mysteriously to a "giant Kohn anomaly", with no explanation of the physics. Platitudes and vague generali-

ties apparently taken from review articles are substituted for a critical evaluation of this field.

When the book discusses subjects in more detail, it often does so uncritically. For example, "Tizze's theory of lambda transitions" is described exhaustively, even though it is almost certainly wrong. As presented by Rao and Rao, it seems to be merely an *ad hoc* hypothesis leading to an infinite specific heat. Most lambda transitions, including the one in superfluid helium, have very singular but finite specific heats, a fact not known to Tizze when he proposed his "theory".

Notwithstanding its faults, this book is a useful source of references and information in the fields it attempts to survey. The numerous figures and illustrations of experimental data are stimulating, although not always clearly explained. I would not recommend this book for a course on the chemistry and physics of solids, as the authors suggest in the introduction. Were it not so prohibitively expensive, *Phase Transitions in Solids* might be worth acquiring as a reference for specialists.

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Recent scientific and technical books

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